NOV -9 1948
©CIB 162308
COPY TO L. OF C. NOV 12 1948
PER.

VOLUME 6 NUMBER 11

10896

# NUTRITION REVIEWS

RECEIVED

## November 1948

Nutrition in Dental Education ..... 321
Catechin and Storage of Ascorbic Acid ..... 324
Influence of Age on Dental Caries Susceptibility ..... 325
Nutritive Value of Marine Zooplankton ..... 327
Goitrogenic Drugs and Thyroid Uptake of Radioactive Iodine ..... 330
Fluorine Content of Normal and Carious Human Teeth ..... 332
Precursors of Niacin ..... 335
Relation of Level of Protein and Pteroylglutamic Acid to Growth and Anemia in the Rat ..... 336
Vitamins and Radiation Injury ..... 336
Effects of Folic Acid Antagonists in Mice ..... 338
Metabolic Effects in Man of Administration of Pituitary Adrenocorticotropic Hormone ..... 339
Glutamic Acid and Intelligence ..... 342
Plasma and Liver Fat Content in Cirrhosis ..... 343
Nutritional Requirements of the Fox and Mink ..... 344
Biochemical Function of Vitamin E ..... 346
Relation Between Ascorbic Acid and Phagocytic Activity of Leucocytes 348
The Hyperglycemic Effect of Thiourea and Related Compounds ..... 349
Notes
Letter to the Editor ..... 351
Survey of Nutrition Research ..... 351
Nutrition Report ..... 351
Protein Hydrolysate for Peptic Ulcer ..... 351

*Published Monthly by*

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK, N. Y.

COPYRIGHT, 1948, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

### *Editor*

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

### *Assistant Editor*

IRENE M. KINSEY, Harvard University, School of Public Health, 695 Huntington Ave., Boston

### *Associate Editors*

HUGH R. BUTT, M.D., Mayo Clinic, Rochester, Minn.
VERNON H. CHELDELIN, PH.D., Department of Chemistry, Oregon State College, Corvallis
WILLIAM J. DARBY, M.D., PH.D., School of Medicine, Vanderbilt University, Nashville
CHARLES S. DAVIDSON, M.D., Thorndike Memorial Laboratory, Boston City Hospital
WENDELL H. GRIFFITH, PH.D., Department of Biochemistry and Nutrition, University of Texas Medical Branch, Galveston
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ESMOND E. SNELL, PH.D., Department of Biochemistry, University of Wisconsin, Madison
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester
M. M. WINTROBE, M.D., PH.D., School of Medicine, University of Utah, Salt Lake City

### *Editorial Committee*

REGINALD M. ATWATER, M.D., American Public Health Assn.
GEORGE R. COWGILL, PH.D., Yale University
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
J. MURRAY LUCK, PH.D., Stanford University
JAMES S. McLESTER, M.D., University of Alabama
HENRY C. SHERMAN, PH.D., Columbia University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
RUSSELL M. WILDER, M.D., University of Minnesota
JAMES R. WILSON, M.D., American Medical Association
JOHN B. YOUMANS, M.D., University of Illinois
H. M. SINCLAIR, M.D., Oxford University
CORRESPONDENT FOR ENGLAND
FREDERICK F. TISDALL, M.D., University of Toronto
CORRESPONDENT FOR CANADA



Subscriptions should be sent to the Nutrition Foundation, Inc., Chrysler Building, New York, N. Y. Rate $2.00 per year in the United States and Canada; $2.50 in other countries.



*Note:* Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved. NUTRICION, a Spanish edition of NUTRITION REVIEWS is published by La Prensa Medica Mexicana, Zacatecas 21, Mexico, D.F. Subscriptions should be sent to this address. Rate $2.00 (U.S. currency) or $10.00 (pesos mexicanos).

# NUTRITION REVIEWS

VOL. 6 NOVEMBER, 1948 No. 11

## NUTRITION IN DENTAL EDUCATION

There are several reasons why nutrition should receive serious attention in the curricula of dental schools. It is necessary that a practicing dentist have a sound background in the fundamentals of nutrition, theoretically, because he should have a broad knowledge of the body as a whole, and practically, if he is to be adequately prepared to take an active role in the utilization and extension of nutritional knowledge in his particular field of public health. Nutrition is not only important in general health but is of especial interest to those caring for the oral cavity first, because the soft tissues of the mouth are among the earliest and most frequent to show signs of nutritional deficiencies (I. Schour and M. Massler, *J. Am. Dental Assn.* **32,** *714, 871, 1022, 1139 (1945)*) and second, because dietary control seems to be one of the most promising ways for the prevention of dental caries. The latter reason seems to be especially important since estimates indicate that restorative dentistry in the hands of the dental personnel presently available can eliminate hardly more than 25 per cent of the caries known to exist in this country (N. Sinai *et al.*, "Public Health Economics," *p. 33, Univ. of Michigan (1946);* L. W. Morrey, *J. Am. Dental Assn.* **32,** *131 (1945)*). Careful dietary counseling of patients with active dental caries has given reductions in the occurrence of new lesions by a magnitude of 60 to 70 per cent (P. R. Howe, R. L. White, and M. D. Elliott, *Ibid.* **29,** *38 (1942)*). More recent and larger experiments by H. Becks and A. L. Jensen (*Read before Int. Assn. Dent. Res., June 19, 1948, publication pending*) gave similar results. The counseling is directed toward a better nutritional pattern, hence is sound from the standpoint of general health. It is easily applicable in the private dental office.

In so far as the dental graduate who is interested in a research career is concerned, his need for an adequate knowledge of the relation of diet to oral health is even greater than that of the man interested in dental practice. Without such training, the investigator will find it difficult, if not impossible, to consider in unbiased fashion his phases of dental problems as they are affected by the role of diet in the differentiation, development, and maintenance of oral tissues. The dental teacher likewise has need of such training if his methods of teaching any dental course to future undergraduates are to include, as they should, a constant recognition and evaluation of the fact that the oral tissues are vitally influenced by diet during development and maintenance and are constantly changing with the metabolic status of the body. Entirely too few good researchers and teachers are available at present to work upon the many unsolved problems in the nutritional field which are related to dental medicine.

The two surveys that have been made to determine the amount of teaching time currently devoted to nutrition in dental schools in the United States and Canada have both indicated that the teaching of nutrition at present is fragmentary. The first survey was made by the Professional Education Section of the American Dietetic Association (*J. Dent. Ed.* **5,** *15 (1940)*), the second was made by D. Hadjimarkos (*J. Dent. for Children* **15,** *10, Second Quarter (1948)*). The final figures of the American Dietetic Association showed thirty dental schools offering some course in nutrition. Hadjimarkos reported only twenty-two dental schools with courses entitled "nutrition," but mentioned that eleven more schools included lectures on nutrition as a part of another course. More important than the

actual number of schools listing themselves as giving attention to nutrition was the amount of teaching time devoted under average conditions and the methods of approach used in teaching. Hadjimarkos found an average of sixteen hours per school devoted to lecture and laboratory instruction. The highest number of hours found was thirty-six. The earlier survey gave total teaching hours for only a few schools, with an average of nineteen hours each. Fifteen of the thirty schools confined the nutrition course to lectures.

Another indication of teaching method lies in the number of dental schools requiring students to take nutritional histories for clinic patients, evaluate these histories, and make necessary recommendations. The American Dietetic Association found only nine out of thirty schools that carried nutrition into the clinic and Hadjimarkos reported twelve out of twenty-two.

No consistent departmental allocation for nutrition was apparent. The first survey found nutrition being taught by teachers of physiological chemistry, medicine, dentistry for children, pathology, oral medicine, biochemistry, diagnosis, preventive dentistry, operative dentistry, and pharmacology. "Nutrition" appeared in the title of only seven out of thirty teachers and one more was called a dietitian. Even though nutrition and biochemistry are subjects so closely interwoven that attempts to separate the two are most difficult, neither survey made any effort to assess the amount of nutrition taught under the heading of biochemistry or physiological chemistry.

The American Association of Dental Schools through its Curriculum Survey Committee ("A Course of Study in Dentistry," *p. 170, Chicago (1935)*) has made recommendations not far in excess of the amount of nutrition actually taught. This committee did not feel that a dentist should be qualified as or be presumed to be an expert nutritionist, nor that he should prescribe definite dietary programs for his patients. They did feel, however, that the dentist should have a working knowledge of general principles in order that he could recognize outstanding dietary faults, and in conjunction with the pediatrician or physician, suggest a corrective diet. The committee recommended that sixteen hours be devoted to "class" work in nutrition and twelve hours to demonstrations and laboratory exercises. A small amount of nutritional material appeared under the committee's recommended course in physiological chemistry (biochemistry), but the total recommendation of time for physiological chemistry was only one hundred and twenty-eight hours.

The result of this limited degree of nutrition teaching in American dental schools has produced approximately the expected effect among our dental practitioners. Most of them, particularly the graduates of the better schools, know the theoretical importance of such nutrients as calcium and vitamin D and will instruct their patients to obtain an "adequate" quantity of these nutrients if the question arises. There is a strong tendency to prescribe calcium and vitamins as pills rather than foods which are rich in these nutrients. Few dentists question their patients routinely about their diet and almost none have either the experience or the desire to take diet histories and to assist their patients in the constructive planning of a good menu. Many dentists feel that prevention of dental caries is "necromancy" and that the highest function a dentist is likely to be able to attain is that of recognizing and filling new lesions of dental caries at the earliest possible moment. It is the impression of the writers that detailed nutritional counseling for dental purposes reaches few persons in this country who do not have sufficiently striking pathologic signs aside from caries to attract real attention, or who are not institutionalized.

A systematic approach to applied nutrition must involve good diet history taking

and analysis and expert nutritional counseling, all matters which require a great deal of fundamental training and practice. If the counselor is to be sure that his recommendations will really be followed, he must assist the patient in solving a great number of problems centering upon food habits, income level, vacation schedule, seasonal availability of certain nutrients, school cafeteria policies, family difficulties, irregular eating hours, and many more. Since the subject is of a fundamental and detailed nature, it belongs logically in the undergraduate curriculum where it can be discussed in terms of and integrated with the various related basic sciences.

Postgraduate courses aid in making the present practitioners of dentistry alert to the opportunity for dietary control of dental diseases, but have the disadvantage that those interested in enrolling do not have sufficient background to utilize short, intensive series of discussions and demonstrations. More thorough courses will be needed than are currently available through dental societies and other organizations. Only a small number of those who take the courses (perhaps chiefly the more recent graduates) may become enough interested to advance to the stage of doing their own nutritional counseling, but all should appreciate the value of this work when they see it done by others to the point that they will seek such advice when they suspect it to be needed.

The foundation of an adequate undergraduate nutrition teaching program should be an extensive course in biological chemistry of two hundred and twenty-five hours or more. Laboratory work in experimental nutrition where the students are responsible for the production and examination of nutritional deficiencies in laboratory animals is a valuable part of this course. Fundamental facts about the pathology of nutritional deficiencies would seem to be covered best in pathology where they can be related to and discussed in terms of other disease syndromes. Around this background, detailed knowledge can be taught to the dental undergraduate about (a) the diagnosis of nutritional deficiencies and of acute and chronic nutritional failure, (b) the relative value of food sources, (c) the taking and evaluation of diet histories, and (d) the development of satisfactory procedures for counseling patients in dietary habits. Teaching of diagnosis of nutritional deficiencies, especially the oral manifestations, can best be done in the courses on dental medicine where they can be adequately related to oral manifestations of other systemic diseases. As a supplement to this, an applied nutrition course is needed in which the first phase would consist of seminars and discussions of food sources, of the methods for the collection and evaluation of diet histories, and of recommendation procedures. The second phase would include demonstrations by the instructor of the actual interview procedure for the collection of diet histories from selected patients, of the evaluation of these histories, and of the possible methods for making satisfactory dietary recommendations based on these appraisals. Then the students must be given adequate opportunity in the clinic, first under supervision and then without, in conducting these procedures themselves. It is difficult to assign a fixed number of hours for such training as the progress of the student, especially in the last phase, depends on his interest in the subject and his ability to obtain the confidence and interest of the patient. The total time should probably be well in excess of fifty hours, thus at least twice the present recommendations of the American Association of Dental Schools.

Current experiments in dental teaching (J. M. Dunning, *J. Am. Dental Assn.* **36,** *90 (1948)*) show how the undergraduate dental curriculum can be freed of much time usually devoted to nonessential subjects. In this way, time can be made available for the

increased program of nutritional teaching just outlined. It seems entirely reasonable to suppose that research will increase the usefulness of nutrition to the dentist and simplify methods of its application. Since the dental schools of the present are training the dentists of the future, nutrition should logically be given an even larger place in the undergraduate dental curriculum than our present concepts would indicate.

JAMES M. DUNNING
JAMES H. SHAW
*Harvard School of Dental Medicine*
*Boston, Mass.*

## CATECHIN AND STORAGE OF ASCORBIC ACID

Conflicting reports from many laboratories concerning the effect of other organic materials in natural foodstuffs upon the utilization or effectiveness of ascorbic acid, have been referred to in earlier reviews (*Nutrition Reviews* **2,** *309* (*1944*)). The reports in general have been characterized by finding that a great variety of compounds of a phenolic nature occasionally affect the mechanical strength and permeability of the capillary vessels of guinea pigs, rats, and human patients.

The term "vitamin P" has been used in the literature in referring to a number of heterogenous physiologic effects and a considerable variety of purified or crude nutrients used in conducting the tests. H. Cotereau, M. Gabe, E. Géro, and J. L. Parrot (*Nature* **161,** *558* (*1948*)) have reported a pronounced physiologic effect of catechin when fed to guinea pigs, in that it permitted tissue storage of ascorbic acid when the latter was supplied at a level of 10 mg. per day. Separate groups of animals receiving the same quantity of ascorbic acid alone, or catechin alone, did not show a comparable ascorbic acid storage during a twenty-three day period.

In a series of previous investigations, this group of workers had postulated an ascorbic acid-sparing activity by phenolic compounds (J. L. Parrot, P. Galmiche, and H. Cotereau, *Compt. rend. Soc. Biol.* **139,** *496* (*1945*)). Subsequent to these observations based on capillary resistance tests in guinea pigs, the authors reported a similar sparing action on the oxidation of ascorbic acid *in vitro* and published a further paper indicating that ascorbic acid dosage in animals was more effective when accompanied by phenolic material (H. Cotereau, M. Gabe, and J. L. Parrot, *Nature* **158,** *343* (*1946*)).

An earlier review (*Nutrition Reviews* **6,** *173* (*1948*)) commented upon reports indicating the presence in fruit juices of materials which stabilize ascorbic acid *in vitro*. The chemical nature of such materials has not been determined.

In the present investigation, adult male guinea pigs which had received a cabbage diet for eleven days were transferred to a basal diet consisting of equal parts of oats and bran which had been autoclaved at 110° with ammonium hydroxide for half an hour. In addition, each animal received daily supplements of thiamine (1 mg.), riboflavin (0.5 mg.), nicotinamide (1 mg.), and twice weekly, 1 ml. of cod liver oil. It is evident that these animals did not have an adequate source of essential nutrients, as shown by reports from several other laboratories.

Four groups were then put on test, as follows: (I) received the basal ration only; (II) received a supplement of ascorbic acid alone (10 mg. per day); (III) received a supplement of catechin alone (1 mg. per day); and (IV) received the above quantities of catechin, plus ascorbic acid.

From the eighteenth day on the ration, animals in groups I and II declined in mean weight, with some deaths between the eighteenth and twenty-third days. Animals in groups III and IV remained almost

stationary in weight, and no deaths were recorded. On the twenty-third day, surviving animals in all groups were killed by bleeding. Autopsy and histologic examination demonstrated the presence of scorbutic lesions in all animals except those of group IV, which received both catechin and ascorbic acid. Animals in groups I, II, and III showed similar, but small, quantities of ascorbic acid in the livers, spleens, kidneys, and adrenals. Animals in group IV were reported to have stored quantities of ascorbic acid four to eight times higher than those in groups I, II, and III.

If confirmed, these observations would have great interest in their implications regarding the role that a number of phenolic substances characteristic of natural food products can play in animal nutrition. There is extensive support in the literature for adopting the authors' suggestion that the term "vitamin P" should be dropped.

## INFLUENCE OF AGE ON DENTAL CARIES SUSCEPTIBILITY

Though a relation between age and susceptibility to dental caries in human beings has been reported for a decade (M. M. Moore, "A Research Conference on the Cause and Prevention of Dental Caries," *p. 88, Good Teeth Council for Children Inc., Chicago* (*1938*)), similar data have rarely been collected for experimental animals.

H. C. Hodge (*J. Dent. Research* **22,** *275* (*1943*)) observed that white rats 1 year old developed carious lesions much more slowly than did weanlings of the same strain maintained on the same caries-producing diet. G. E. Braunschneider, H. R. Hunt, and C. A. Hoppert (*Ibid.* **27,** *154* (*1948*)) have begun investigations for this purpose with representatives of their ninth generation of white rats which were bred for caries susceptibility. The caries-producing ration used in these studies was that described by C. A. Hoppert, P. A. Webber, and T. L. Canniff (*Ibid.* **12,** *161* (*1932*)) and was composed of: 66 per cent coarsely ground polished rice, 30 per cent whole milk powder, 3 per cent alfalfa leaf meal, and 1 per cent sodium chloride. The ration which prevented or greatly retarded the development of carious lesions consisted of the above ration with the coarsely ground polished rice completely replaced by a finely ground rice. A third ration in which the coarsely ground rice was completely replaced by a more finely ground material, commercial rice flour, was found to be still less conducive to the development of carious lesions, but in this strain of highly caries susceptible rats, occasional rats were found to develop lesions even on this diet. In early experiments, the second diet was used for breeding purposes and to maintain the young animals until the beginning of the experimental period when feeding of the coarse ration was begun. Fifteen animals from the eighth generation which had already developed carious lesions were used as the breeders in this experiment. It was planned to compare the susceptibility to tooth decay of animals which were 35, 100, or 150 days of age at the time the coarse ration was first fed. Eighty-six rats, 35 days of age, which had been previously maintained on the second ration were fed the coarse, caries-producing ration until carious lesions could be observed when the rats were examined under a strong light by the unaided eye. The mean length of time elapsing between the beginning of the experiment and the observation of carious lesions ("caries time") was twenty-nine and three-tenths days with a standard deviation of nine and three-tenths days. Of a second group of 26 rats which had been maintained on the finely ground rice ration after weaning, 11 developed carious lesions at an unmeasured time less than one hundred days, while the remaining 15 had a mean "caries time" of fifty-two and three-tenths days. Thus the

susceptibility of these rats to dental caries was so high that caries-free animals 100 or 150 days of age could not be produced through this experimental procedure. Therefore for the period from weaning to 100 or 150 days for the rats in these two groups, the rice flour ration was used. The mean "caries time" for the group of 49 rats fed the coarse caries-producing ration from 100 days of age was fifty-seven and six-tenths days with a standard deviation of twenty-nine and six-tenths days. Two of the 43 rats in the group which were not fed the coarse ration until 150 days of age had "caries times" of two hundred and twenty-three and two hundred and eighty-three days, respectively, while the mean "caries time" of the group was fifty-two and five-tenths and the standard deviation was fifty-five and nine-tenths days. Mathematical evidence suggested the exclusion of these 2 aberrant individuals. When this was done, the mean "caries time" for this group was forty-two and eight-tenths days with a standard deviation of thirty-four and three-tenths days. The differences between the mean "caries times" of the 35 day old group and the 100 day old group and between the mean "caries times" of the 35 day old group and the 150 day old group were highly significant (at the 1 per cent level). The difference between the mean "caries times" of the 100 day old and the 150 day old group was significant at the 5 per cent level but not at the 1 per cent level. Thus the 35 day old group at the beginning of the caries-producing dietary regimen had a definitely higher caries susceptibility than rats which were 100 or 150 days old when the coarse, caries-producing ration was begun. Probably the 150 day olds had a slightly higher caries susceptibility than the 100 day olds. These data indicate the need to control carefully the age of animals used in dental caries experiments.

The three ages chosen for this investigation are widely spread for an animal with a life span so short as that of the white rat. One cannot help wondering what the caries susceptibility is at other ages and what shape a curve of caries susceptibility versus age would assume if accurate data were available at small time intervals from weaning at 18 or 19 days up to early senility. Also, is there any critical age after which the caries susceptibility is reduced, or has a steady maturation process of the tooth substances occurred after eruption which has rendered the teeth less susceptible to tooth decay? If the latter should be demonstrated to be true can the rate and completeness of maturation of the teeth be influenced by dietary alteration? The caries attack rate is highest in human beings in the period between early adolescence, when there is a rapid replacement of the deciduous teeth by the permanent dentition, and early adult life. This is, of course, a period of rapid growth and development and of high nutritional requirements. After 25 years of age, the frequency of new carious lesions decreases sharply until a low level of new carious lesions per year is reached which continues unchanged throughout the rest of the life span (F. Hollander and J. M. Dunning, *J. Dental Research* **18,** *43 (1939)*). The situation in the rat cannot be directly or completely related to that in man since the rat has only one dentition which is fully erupted by about the fortieth postnatal day (M. M. Hoffman and I. Schour, *Am. J. Orthodontics* **26,** *854 (1940)*). However, the progress of carious lesions in the permanent dentition in man is roughly comparable to that in the molars of the rat under the circumstances of the regimen described by Braunschneider *et al.* It is of considerable importance to work out the caries susceptibility curve for experimental animals in complete detail as one of the possible methods for studying the relation of metabolic processes to susceptibility to tooth decay. The unfortunate limitation of the above experiment was the use of the coarse particle diet which has been shown to produce carious lesions at least partially through

fracturing the tooth substances. The procedure for producing carious lesions without any previous mechanical injury which has been described recently would have a great many advantages for a continuation of this experiment in the detail needed, especially since mechanical injury has never been demonstrated to precede carious lesions routinely in human beings (*Nutrition Reviews* **6,** *196 (1948)*).

## NUTRITIVE VALUE OF MARINE ZOOPLANKTON

The waters of the earth have long been recognized to contain a larger amount of living organisms than inhabit the land because of their greater extent. This food source has been drawn upon by man chiefly in the form of fish, mollusks, the higher crustacea, the total of which constitute in volume and variety only a small portion of the available organisms. Almost untouched as human food are the various lower forms which are commonly referred to as the phytoplankton and the zooplankton. A portion of these lower organisms represents the food of the higher aquatic animals and thus contribute to the formation of the present edible marine harvest, but the greatest majority die after normal life spans and contribute to the organic residues in the ooze at the bottom of the ocean.

G. L. Clarke and D. W. Bishop (*Ecology* **29,** *54 (1948)*) have reviewed the literature to collect the available data on the concentration of marine plankton in various parts of the world and the use which has been made of these lower organisms in human nutrition. In addition, they have contributed further data on the distribution of plankton and the ability to collect it and have conducted a series of preliminary experiments with rats to determine the nutritional value of various samples of plankton. Their studies were limited primarily to zooplankton because the small size of the phytoplankton (plant forms) and the nannoplankton (dwarf species) made collection in large quantities difficult. In part, their studies were conducted to determine to what extent zooplankton could be obtained and used as an emergency food to augment the quantity of food stored on life rafts.

Previous studies on the palatability and availability of plankton as a source of nutrients for human use are practically nonexistent. Clarke and Bishop (*loc. cit.*) cited three instances where plankton in some form had been used for human food. Along the northern coast of China, a dish is prepared from plankton which is said to serve as a "main accessory food" for the poor people during the winter. Various kinds of small marine crustacea are eaten in the Scandinavian countries. In a popular article, T. Heyerdahl (*Reader's Digest* **51,** *102 (Nov. 1947)*) described the collection of plankton and its consumption by a 6-man expedition during an experimental raft drift eastward from Peru. No information was given on the type or amount of plankton consumed per man per day, the amount of other food consumed, and the amount of fresh water consumed with the plankton. In any discussion of the availability of plankton for food, it is necessary to define with a reasonable degree of accuracy the distribution of organisms composing the particular sample under consideration, as the nutritive properties may vary with the relative distribution of species. The kind of organism which dominates the plankton may vary within a few miles or less but may be uniform for large areas (M. Sears and G. L. Clarke, *Biol. Bull.* **79,** *321 (1940)*). In any area, the interval of time necessary for an appreciable change may be as short as a few days or as long as several weeks. Frequently a diurnal variation was observed as a result of migration of some types of organisms to and from the surface of the sea daily

(Clarke, *Ibid.* **67,** *432 (1934)*). The animals which were usually most abundant were the crustacea and particularly the copepods, although euphasids predominated occasionally. In some cases, medusae, salps, siphonophores, sagittae, and other soft-bodied animals comprised more than 90 per cent of the zooplankton. At certain seasons, the phytoplankton outweighed the zooplankton. Species with long spurs and chain forms were often retained in a coarse-meshed plankton net intended for zooplankton in sufficient numbers to prevent filtration. Diatoms also reduced the filtering efficiency nearly to zero on certain occasions.

The composition of samples of plankton which were obtained in November for chemical analyses consisted of about 95 per cent copepods (largely *Centropages* sp.), with small percentages of other crustacea, worms, pelecypods, and fish larvae. Each sample was divided into three portions. The first portion was drained, weighed wet, and weighed dry before analysis. The second was drained, washed with fresh water, drained, weighed wet, and weighed dry. The third was drained, excess water squeezed out by hand through netting with moderate pressure, weighed wet, and weighed dry. Analyses of the dried matter were made for protein, fat, total ash, insoluble ash, chlorine, sodium, phosphorus, potassium, calcium, magnesium, sulfur, and carotene. Carbohydrate contents were determined by difference. Protein (N x 6.25) for the three aliquots was 52.6, 59.2, and 56.2 per cent, respectively. Thus the protein content of the samples was relatively high. Fat values were 1.4, 4.2, and 4.1 per cent, respectively, and total ash were 32.9, 19.4, and 24.1 per cent, respectively. Of the elements analyzed, sodium and chlorine were most abundant. Sodium chloride was reduced from 22 to 14 per cent by squeezing and to 9 per cent in the portion which had been washed. The ratio of magnesium to potassium was much higher than in the body fluids of the warm-blooded animals.

An approximate calculation was made of the effect of a plankton diet on the water balance of the body on the basis of the chemical composition of the squeezed plankton. Exact values were not possible without human trials. An approximation of the volume of water required for the renal excretion of the solution in 1 g. of dried plankton was found to be about 5.7 ml. Since squeezed plankton contained 86 per cent water, about 6 ml. of water would be taken in per gram of dry matter in the plankton. Theoretically, this should be adequate for the renal excretion of the contained salts without additional drinking water. The imbalance of magnesium and potassium in relation to their distribution in the body fluids of warm-blooded animals might alter this calculation.

If all organic components in the squeezed plankton were utilizable as energy sources, a maximum of 4 calories per gram of dry weight would be available. Clarke and Bishop (*loc. cit.*) estimated from studies on zooplankton distribution that about 2400 ml. of drained plankton could be obtained on a life raft in twenty-four hours under normal conditions of drift, etc. which would provide about 800 calories if completely utilizable. Unpublished studies based on the consumption of sea water which has a similar mineral distribution to plankton quoted by these investigators suggested that an excess of 1200 ml. of drained plankton could not likely be consumed per man without harmful effects in the form of vomiting and diarrhea.

Preliminary experiments were conducted to determine whether weanling rats could derive any nourishment from plankton when either one third or two thirds of the usual stock ration was replaced by plankton. Previous studies indicated that 13 g. of this stock ration were required by weanling rats to permit the maximum growth possible when this stock ration was used as the sole source of nutrients. Diet mixtures were prepared by mixing stock ration and plankton which had been squeezed with moderate pressure to re-

move excess sea water. The squeezed plankton which was frozen and stored until used contained 14 per cent of dry matter by weight. In the first experiment, two thirds (8.5 g. per day) of the amount of stock ration required by weanling rats for maximum growth was fed to each of the rats in the first group. A second group of 12 rats was each fed daily this amount of stock ration mixed with 32 g. of wet, squeezed plankton which supplied 4.5 g. of dry matter. In this way the individual rats of the second group had access to 13 g. of solids per day. A third group of 12 rats was fed the stock ration ad libitum as controls. Although growth of the rats receiving one third of their diet as plankton was more irregular than that of the controls, the average rate of growth for these rats was essentially the same as that of the controls during the thirteen day experimental period, about 36 g. for the experimental rats, and 39 g. for the controls. It is important to note that these weight increases do not represent the maximum growth responses possible for weanling rats under the most favorable dietary regimens. This would suggest that the stock ration itself might not be nutritionally adequate. The first group of rats which received 8.5 g. of stock ration daily without plankton gained, on the average, about 26 g. Thus the plankton had been able to replace one third of the stock ration reasonably satisfactorily except for some irregularity in weight responses.

In the second experiment, the first group of 9 rats was fed on a diet of one-third stock ration mixed with two-thirds squeezed plankton, i.e., 4.5 g. of stock ration and 60 g. of plankton per day. The 10 rats in the second group were fed 4.5 g. of stock ration daily. The rats in the first group consumed only about two thirds of the mixture offered daily; these rats did not grow but did maintain their original body weight during the seven day period. The rats in the second group lost considerable weight during the same period. Under the circumstances, the plankton definitely had contributed to the nutrition of the rats. The refusal or the inability of the rats to consume all the diet offered undoubtedly was partially responsible for the lack of growth. Purely on the basis of volume, the weanling rats may have been unable to eat in excess of two thirds of the amount offered. An interesting point to test in this regard would be whether a longer experimental period would have permitted the rats to adjust themselves to this diet sufficiently to permit some growth.

When rats were offered wet, squeezed plankton as the sole source of nutrients, they began to lose weight immediately and eventually died. Weanling rats on this regimen died within four to five days, while adult rats survived eighteen to nineteen days. Starved adult rats of equivalent initial weights given adequate water lived for a maximum of thirteen days. Less than 60 g. of wet, squeezed plankton were consumed daily by adult rats, i.e., about 7 g. of solids, in contrast to a normal consumption of 20 g. of stock ration by adult control rats.

Tests made with fresh plankton did not reveal any deleterious condition resulting from frozen storage. Some portions were squeezed as before, others washed with distilled water and others washed and cooked twenty to thirty minutes. These were supplied as the sole source of nutrients to groups of young rats. The daily consumption of washed plankton by weanling rats was considerably better than of fresh, unwashed plankton or of washed, cooked plankton.

The rats which were fed whole or partial plankton diets were found to consume on an average about the same quantity of water as the controls in addition to the 86 per cent of water in the squeezed plankton. For example, rats fed plankton consumed only 86 to 90 g. of water from the water bottles for every 100 g. of wet plankton.

Autopsies on plankton-fed animals indicated few abnormalities. The kidney appeared normal. The most consistent finding was a marked accumulation of fecal ma-

terial in the cecum and colon. The cause of this congestion was not known but possibly could be attributed to the general weakness and loss of muscle tone in the period before death. It was unknown whether this congestion was a contributory cause of death.

Since the maximum concentration of zooplankton encountered by Clarke and Bishop (*loc. cit.*) was in the neighborhood of 5 ml. per cubic meter of sea water, the amount of water necessary to be filtered to obtain appreciable quantities would be large. The feasibility of filtration for commercial purposes was not discussed by these investigators. As an emergency food supplement, the plankton appeared to have definite possibilities even if the rations on a life raft could be extended by only 10 or 15 per cent by the use of plankton.

As a food, the definite limitation of plankton appears to be the high mineral content and in particular the high magnesium:potassium ratio. However, the protein content is high, sufficiently high indeed to make a good protein supplement for human or livestock use if the protein is of good quality and if the cost of filtration, reduction of salt content, and dehydration are not prohibitive. The value of plankton as an over-all nutrient seems fairly satisfactory under the conditions of the described experiments. The fact that plankton alone does not support growth and development in weanling or adult rats is not a serious condemnation of it as there are few single food materials known which in themselves are sufficiently complete to permit even slight growth without supplementation or to maintain adult animals.

## GOITROGENIC DRUGS AND THYROID UPTAKE OF RADIOACTIVE IODINE

The rate and degree of thyroid absorption of tracer doses of radioactive iodine have been the basis of various clinical tests for estimating the degree of activity of the thyroid gland in incorporating iodine in the synthesis of thyroxine. In patients with increased or decreased functional activity of the thyroid gland, these various technics have given results which parallel reasonably well other indexes of thyroid activity. Recent experiments in animals by R. W. Rawson *et al.* (*J. Pharmacol. Exp. Therap.* **93,** *240 (1948)*) indicate that while decreased production of thyroxine and diminished uptake of iodine by the thyroid are usually associated phenomena, conditions may be produced in which uptake of iodine may be normal or even supernormal at a time when thyroxine production appears to be greatly diminished. These results were observed in rats and in chicks during a study of thyroid-iodine-uptake after the animals had been fed one of a number of goitrogenic drugs for a period of two weeks.

It had been known (R. W. Rawson, J. F. Tannheimer, and W. Peacock, *Endocrinology* **34,** *245 (1944)*, A. L. Franklin, S. R. Lerner, and I. L. Chaikoff, *Ibid.* **34,** *265 (1944)*) that goiters produced in the rat by continued administration of thiouracil, collected less radioiodine than did the controls. But, while the former group had observed that rat thyroids made goitrous with potassium thiocyanate trapped a greater proportion of radioiodine than normal, the latter had found that surviving sheep thyroid failed to collect radioiodine from a Ringer-bicarbonate medium in the presence of potassium thiocyanate while in the absence of the latter compound it not only absorbed iodine but converted it into diiodotyrosine or thyroxine. Using the same *in vitro* technic, they had observed another apparent paradox, in that in the presence of thiourea, thiouracil, or allylthiourea in the medium, instead of potassium thiocyanate, uptake of radioiodine occurred but it failed to be converted into thyroxine or its precursor.

Rawson and his associates (*loc. cit. 1948*) made rats and chicks goitrous by feeding thio-

uracil, 6-propylthiouracil, 6-benzylthiouracil, 2-aminothiazole, 5-aminothiadiazole-2-thiol (TC-68), 3-(phenylaminomethyl) thiazolidine-2-thione (TC-105), and potassium thiocyanate. (It is to be recalled that the goitrogenic effect of potassium thiocyanate is prevented by the co-administration of iodine.) Four hours before the animals were killed, radioiodine was administered. The thyroids were removed and then analyzed for their contents of radioiodine.

In rats, thiouracil, TC-68, and TC-105 were equally goitrogenic and all of them produced a block in the uptake of radioiodine. TC-105 exerted a somewhat more effective block than did the other two. Aminothiazole, at the same dose level as the other three was less goitrogenic and produced a much less effective block to the uptake of radioiodine. Potassium thiocyanate proved to be only slightly goitrogenic, and iodine uptake per unit of thyroid tissue was but mildly inhibited by this agent.

In chicks quite different results were obtained with some of these same drugs. TC-105 and TC-68, which in the rat effectively inhibit thyroid uptake of iodine, were found to increase iodine uptake in the goiters of chicks. When expressed on the basis of iodine uptake per milligram of thyroid tissue, the increase came to 22 per cent and 46 per cent above that found in the control animals. Potassium thiocyanate proved to be an effective goitrogen in the chick, but goiters thus produced manifested no block to the collection of iodine. In fact, these goiters collected 17 per cent more iodine per unit mass of tissue than that collected by the controls. Aminothiazole exerted little goitrogenic effect in the chick but increased iodine uptake in the thyroid by 81 per cent. Goiters produced in the chick by thiouracil, propylthiouracil, and benzylthiouracil handle iodine as do goiters produced in the rat by thiouracil, that is, there occurs an effective block to the uptake of iodine.

It appears then, that some agents which are goitrogenic for both the rat and chick, bring about this result by different mechanisms. It seems, at least, that synthesis of thyroxine may be sharply inhibited by certain drugs which at the same time exert no inhibitory effect upon uptake of iodine by the thyroid. Another possibility is that some of the goitrogens may act by exerting a potentiating influence upon the animals own thyrotropic hormone. Some evidence to support this concept has been presented by A. Albert *et al.* (*Endocrinology* **40,** *299, 303, 361 (1947)*).

In line with these considerations, the carefully conducted experiments of D. A. McGinty *et al.* (*J. Pharmacol. Exp. Therap.* **93,** *246 (1948)*) appear to be significant. These workers studied again in rats and in chicks the same antithyroid drugs mentioned above. This time, however, they were interested in changes in thyroid-iodine-uptake within the first twenty-four hours following a single subcutaneous injection of the agent into a normal animal. Here, obviously, a goitrogenic effect was not involved.

In both the rat and the chick some inhibitory effect of thyroid-iodine-uptake was demonstrated with all of the drugs used. What seems significant is the fact that the intensity and duration of the inhibition of iodine uptake exerted by the various agents are frequently not at all related to the known goitrogenic capacities of these same drugs. Thus, "it becomes apparent that the goitrogenic action of these agents is not entirely dependent upon the inhibition of iodine collection."

Clearly, further work along such lines will lead to important discoveries relative to the mechanisms of action of antithyroid drugs and to the physiology and biochemistry of thyroid function in general.

## FLUORINE CONTENT OF NORMAL AND CARIOUS HUMAN TEETH

W. D. Armstrong and P. J. Brekhus (*J. Dental Research* **17,** *393 (1938)*) described an investigation in which the fluorine content of enamel from the intact teeth of 3 individuals who were not known to have been exposed to water supplies bearing fluorides was significantly higher than the fluorine content of the enamel from the carious teeth of the same individuals. In addition, the mean fluorine content of enamel from the sound teeth of 10 persons was significantly higher than the mean fluorine content of the samples of enamel from the carious teeth of 8 persons. Only one of these cases contributed both sound and carious teeth. These data have been widely considered as one of the strong contributions of indirect evidence in favor of the use of fluorine supplementation of public water supplies as a possible method for the reduction of the present high incidence of tooth decay. The range of fluorine levels in enamel from sound teeth was from 0.0073 to 0.0167 per cent with a mean of 0.0111 per cent in a total of forty-six analyses; the range of fluorine levels in enamel from carious teeth was from 0.0049 to 0.0087 per cent with a mean of 0.0069 per cent in a total of thirty-nine analyses. The means for fluorine content of dentin from sound and carious teeth were 0.0169 and 0.0158 per cent, respectively, as determined by one sample of each prepared by pooling 50 mg. quantities of dentin from each of twenty-six teeth.

Less widely discussed values for the fluorine content of sound and carious enamel were presented by T. Ockerse (*J. Dental Research* **22,** *441 (1943)*) who made a similar survey in a nonfluoride area in South Africa where about 99 per cent of the inhabitants had had some evidence of tooth decay. In this case, the mean fluorine content for the enamel from ten sound and three carious teeth was found to be 0.0153 per cent for each; the mean fluorine content of the dentin from nine sound and five carious teeth was 0.0268 and 0.0252 per cent, respectively. These values were considerably lower than those determined by the same investigator in an area where the water supply contained 1.5 parts per million of fluorine. The mean fluorine content of enamel from six sound teeth of individuals residing in this region was 0.0445 per cent with a range of 0.0150 to 0.0750 per cent. The mean fluorine content of dentin from eight sound teeth was 0.0755 per cent with a range of 0.0130 to 0.1700 per cent. Unfortunately, no analyses were made of the fluorine content of enamel and dentin from carious teeth of individuals residing in the fluoride region. Numerous other investigators have reported comparable values for the fluorine content of enamel and dentin of sound teeth from individuals exposed to fluoride waters, but no values have been reported for carious teeth of these individuals. In view of the present importance for a detailed, unbiased consideration of the possible role played by fluorine in the maintenance of normal teeth, these contradictory data on the fluorine content of carious and normal teeth appear to be much more limited than is merited.

An investigation to elaborate on the fluorine content of human teeth and "to attempt to explain the role of fluorine as a caries-preventive element" has been conducted by F. J. McClure (*J. Dental Research* **27,** *287 (1948)*). Using the modifications of W. D. Armstrong (*Ind. Eng. Chem. (Anal. Ed.)* **8,** *384 (1936)*) and of McClure (*Ibid.* **11,** *171 (1939)*) of the original titration technic (H. H. Willard and O. B. Winter, *Ibid.* **5,** *7 (1933)*), fluorine analyses were made on two hundred and sixty-two sound and two hundred and forty-eight carious teeth collected from 91 individuals. The mean fluorine content of the enamel of these sound and carious teeth was 0.0106 and 0.0102 per cent, respectively, while the mean fluorine content of the dentin of sound and carious teeth was 0.0241 and 0.0225 per cent, respectively. The 91 individuals represented in this study

were divided geographically into three groups. The first group constituted a rather random sample whose extractions were performed mostly in Washington, D. C. Their residence histories as far as they were available varied greatly. The second group of 14 individuals had presumably been continuous residents of McDuffie County, Georgia, where the caries prevalence is somewhat low and where there is no evidence of an unusual fluoride exposure. Though the caries experience was low, no significant difference was observed in the average fluorine contents of the enamel and of the dentin from sound and carious teeth of these individuals when these values were compared with those values for the first group. The third group was composed of 44 persons residing in north central Illinois "whose residence history was not established and remains quite uncertain." Although the teeth of these individuals were collected in a geographic area which has a history of moderate fluoride waters (1.5 to 2.5 p.p.m of fluorine), no elevation in enamel and dentin fluorine was disclosed in this analysis. None of the teeth of the individuals in the three groups had a sufficient preeruptive exposure large enough to produce mottled enamel. The group of 33 individuals, mostly from Washington, D. C., were studied most extensively; 19 provided both sound and carious enamel. This group probably represented a widespread difference in fluorine exposure during life, certainly greater difference than either of the other two groups who had resided in somewhat more isolated circumstances. The fluorine content of the enamel of sound and carious teeth from 16 different individuals was 0.0100 (0.0067 to 0.0173) and 0.0097 (0.0054 to 0.0154) per cent, respectively, while the fluorine content of the dentin for sound and carious teeth from 19 different individuals was 0.0214 (0.0133 to 0.0319) and 0.0219 (0.0119 to 0.0327) per cent, respectively. These small differences were found by statistical evaluation to be entirely insignificant. Thus, in this comparison on the basis of sound and carious tooth compounds from the same individuals, there is no indication that differences in fluorine content could be related to the carious or noncarious status of the tooth.

An attempt was made to determine whether the different developmental and calcification characteristics of the various functional types of teeth as well as possible variations in the time of exposure to fluorides might have introduced variations in the amount of fluorine acquired by the teeth as they developed and calcified. Teeth were classified into three types: (a) consisting of first incisors, second incisors, and canines; (b) consisting of first premolars and second premolars; and (c) second molars. Fluorine analyses were tabulated in accordance with the type of tooth. In all, ten comparisons of the fluorine content of sound and carious enamel and dentin from the same person were possible in type (a) teeth, six in type (b), and two for type (c). Again the comparison between sound and carious teeth indicated that there was no statistically significant difference in the fluorine content of either the enamel or the dentin. When the fluorine content of sound dentin and sound enamel was compared for canine teeth versus incisors no statistically significant difference was observed. Again, when the fluorine content of sound dentin and sound enamel was compared for premolars versus combined incisors and canines, no significant difference was observed. When the fluorine content of carious dentin and carious enamel was compared for second molars versus premolars no significant difference was observed.

Since no significant difference in the fluorine content of sound and carious enamel and dentin was found to be a general rule among the individual dentitions studied, there seems to be considerable reason to believe that the caries experience of teeth from individuals selected at random is not entirely dependent upon their fluorine content. The fact that there is no variation in the fluorine content

of different functional, developmental, and anatomic types of teeth has brought to light no significant variation in either dentin or enamel. This strongly suggests that the different caries susceptibility of various types of teeth is not due to alterations in fluorine induced by different function, developmental time, or anatomic structure. The presence of fluorine in the drinking water supplies during tooth development has been demonstrated by various investigators to result in a much higher fluorine content in both enamel and dentin than the highest amounts observed in these studies. These investigations by McClure do not necessarily mean therefore that the reduced caries experience observed in the fluoride regions is unrelated to the increased amount of fluorides in tooth structures developed in these areas.

One possible source of error described by McClure was the difficulty in determining whether a tooth was in a state of active caries susceptibility. Under the conditions of the experiment, the teeth were described as carious when a cavity or filling was found therein. An unfilled cavity was indicative of past or present carious activity while a filling was essentially a record of previous caries experience. Between the time of restoration or the cessation of active caries and the date of tooth extraction, fluorine might have been acquired or lost posteruptively. Therefore, the designation of "carious" throughout referred to a tooth which had had a carious lesion at a prior undetermined period in its life history and may or may not have been actively carious at the time of extraction and use in this study.

Likewise the definition of "sound" tooth had a similar limitation, in that the early lesions of dental caries might not have been detected. The "sound" tooth might be in a definite state of high caries susceptibility at the time it was extracted but might have been in that state for an insufficient period to produce what can be clinically diagnosed as a carious lesion. This inability to diagnose whether a cavity is in active state of caries development or whether an apparently sound tooth actually has been altered by biochemical lesions preceding visible carious lesions is one of the big limitations of dental research at the present time. As in this study, the fluorine content determined could be only that of a tooth and its components at the time of extraction and need not have been representative of the fluorine content of the enamel and dentin at the time of the active caries development.

These are sources of error over which an investigator has little or no control. The much more serious criticism of this investigation is the lack of data on the past residential histories of the subjects. For example, were the subjects from north central Illinois continuous residents in communities where the public water supplies contained 1.5 to 2.5 p.p.m. of fluorine during their periods of tooth development or were they recent arrivals in the community? If they had lived there during tooth development, what is the explanation of the much lower fluorine content of their enamel and dentin than those values determined by Ockerse (*loc. cit.*) and other investigators in communities where the water supply contained 1.5 p.p.m. of fluorine? Or had there been a period of nonresidence in a fluoride region during which the fluorides had decreased in tooth substance. The data presented by McClure demonstrate, even with the above limitations, that differences in the fluorine content between the enamel from the carious and sound teeth from any individual are extremely small, if not nonexistent, and must be interpreted with caution. Certainly in their present status such differences cannot be used as arguments in favor of artificial fluorination of public water supplies.

Two of the most valuable problems in this field at the present time would be to determine whether there is a difference between the fluorine content of the enamel and dentin from sound and carious teeth of individuals residing in fluoride regions during tooth development and whether there is a decrease of

the fluorides in enamel and dentin of teeth developed during residence in fluoride areas when exposure to the fluoride waters ceases posteruptively. Critical answers to these problems would prove to be significant additions to the knowledge of fluorides and their action and would augment McClure's data considerably.

## PRECURSORS OF NIACIN

A recent review (*Nutrition Reviews* **6,** *50* (*1948*)) has summarized the work, principally with mutant strains of Neurospora, which has led to the discovery of two crystalline nitrogenous compounds, as well as the identification of kynurenine, as possible precursors of niacin.

Further reports from two laboratories indicate that one of the above compounds (produced by strain 4540 and possessing niacin activity for strain 3940) is 3-hydroxyanthranilic acid. This surprising relation between an aromatic (benzenoid) compound and a pyridine derivative was deduced by H. K. Mitchell and J. F. Nyc (*Proc. Nat. Acad. Sci.* **34,** *1* (*1948*)) in attempting to understand the mechanism of the conversion of kynurenine into niacin. These workers after preparing a number of derivatives of niacin were led to postulate the formation of 3-hydroxy kynurenine as a possible precursor of xanthurenic acid in metabolism. Consideration of this hypothetical compound suggested the possibility that 3-hydroxyanthranilic acid might in turn be formed from it by oxidation of the side chain.

Using synthetic 3-hydroxyanthranilic acid, Nyc and Mitchell (*J. Am. Chem. Soc.* **70,** *1847* (*1948*)) found that the mutant strain 65001, which can convert tryptophan to niacin, utilized 3-hydroxyanthranilic acid approximately as effectively as niacin. That niacin was actually formed from 3-hydroxyanthranilic acid was indicated by the latter's stimulation of growth of strain 3416, which responds only to the vitamin or its amide.

Proof of the identity of 3-hydroxyanthranilic acid and the $C_7$ acid produced by strain 4540 has been furnished by D. Bonner (*Proc. Nat. Acad. Sci.* **34,** *5* (*1948*)), who compared the melting points, absorption spectrums, and physiologic activity of the two compounds. The naturally occurring compound was previously reported to be a pyridine derivative (Bonner and G. W. Beadle, *Arch. Biochem.* **11,** *319* (*1946*)) but it appears that this conclusion was reached chiefly from a consideration of the physical properties of the compound.

Thus it appears that 3-hydroxyanthranilic acid is involved in niacin synthesis in Neurospora. However, it is pointed out (Bonner, *loc. cit.*) that evidence for the position of the former as a direct precursor of the vitamin, as well as the validity of the complete scheme of conversion of anthranilic acid $\rightarrow$ indole $\xrightarrow{+\text{ serine}}$ tryptophan $\rightarrow$ kynurenine 3-hydroxyanthranilic acid $\rightarrow$ unknown compounds $\rightarrow$ niacin, are still open to question. Chief objections to the scheme are (1) the existence of at least one mutant (75001) which can utilize anthranilic acid, tryptophan, or kynurenine for growth but which is unaffected by 3-hydroxyanthranilic acid or niacin; (2) the inactivity of kynurenine as a precursor of N′-methylnicotinamide in the rat; and (3) the inability of kynurenine or 3-hydroxyanthranilic acid to produce rat growth in the absence of tryptophan and niacin. Further work seems necessary to establish the specific role of each compound which effects the synthesis of niacin in different species.

## RELATION OF LEVEL OF PROTEIN AND PTEROYLGLUTAMIC ACID TO GROWTH AND ANEMIA IN THE RAT

An extremely high incidence of folic acid deficiency in rats fed diets low in protein as compared with that in animals receiving ample protein was noted in 1946 by A. Kornberg (*J. Biol. Chem.* **164,** *203 (1946)*) and by Kornberg, F. S. Daft, and W. H. Sebrell (*Science* **103,** *646 (1946)*). In a subsequent note dealing with this effect, Daft (*Federation Proceedings* **6,** *405 (1947)*) found that weanling rats fed a diet containing 4 per cent of casein as the sole source of protein became anemic, leukopenic, and granulocytopenic. The level of the circulating white cells in such animals was increased by treatment with folic acid, refined liver extracts (15 units per milliliter) of the type used in the treatment of Addisonian pernicious anemia, or by administration of ten essential amino acids in amounts sufficient to correct the protein deficiency. The effect of the liver extract was not due to its content of pteroylglutamic acid, which was very low. Details of this latter study have not appeared, but again emphasize the appearance of symptoms on low protein diets which are corrected by folic acid.

The level of purified protein necessary to prevent appearance of symptoms of folic acid deficiency in rats fed a diet lacking in this vitamin has now been determined by O. Shehata and B. C. Johnson (*Proc. Soc. Exp. Biol. Med.* **67,** *332 (1948)*). The ration was of synthetic type, containing purified casein, glucose, salts, corn oil, synthetic vitamins (except folic acid), and sulfathalidine. The casein was varied from 5 to 30 per cent at the expense of glucose. Groups of 5 weanling rats each were fed these rations for six weeks. Starting with the third week, records of blood hemoglobin and red blood cell counts were kept in addition to weight records.

The diet with 5 per cent casein was just sufficient to maintain weight in the animals over the six week period; no gains in weight were made. This was true whether or not the diet was supplemented with folic acid. Optimal growth was achieved with 20 per cent casein, and levels of 15 and 10 per cent produced intermediate rates of gain. Results of hemoglobin determinations were different. At the end of six weeks, hemoglobin levels in rats fed 5, 10, 15, 20, and 30 per cent of casein averaged about 8.5, 10.3, 11.3, 12.4, and 13 g. per 100 ml. of blood, respectively. In contrast, animals receiving 5 per cent of casein plus pteroylglutamic acid showed an average hemoglobin level of 12.9 g. per 100 ml. of blood. Thus, the deficiency in hemoglobin production, but not the growth deficiency, observed on the low protein diet was completely alleviated by folic acid. Para-aminobenzoic acid was not effective, since it was present in the ration.

These results would appear to support the suggestion of Daft (*loc. cit.*) that, under appropriate conditions (i.e., high protein rations) some synthesis of pteroylglutamic acid normally occurs in the rat, and that essential amino acids may be spared to some extent when adequate amounts of this vitamin are administered. The magnitude of any sparing action, however, must not be great, since no significant differences in weights of animals on the low level of casein were observed whether or not pteroylglutamic acid was present.

## VITAMINS AND RADIATION INJURY

The general use of x-ray therapy in cancer, and the possible use of "atom bombs" in warfare, have served to increase interest in the mechanism by which radiation sickness is produced, and in its treatment.

In a study of agents which influence exper-

imental radiation injury, A. Goldfeder, L. Cohen, C. Miller, and M. Singer (*Proc. Soc. Exp. Biol. Med.* **67,** *272 (1948)*) investigated the effects of dietary supplements of folic acid and of pyridoxine on the susceptibility of mice to radiation injury. These two vitamins were selected for investigation because the former is reported to be of clinical value in preventing nausea in x-ray-treated cases (L. D. Scott and G. J. Tarleton, *Radiology* **47,** *386 (1946)*; L. M. Shorvon, *Brit. J. Radiology* **19,** *369 (1946)*), whereas the latter is of value in sprue and in treatment of leukopenia (*Nutrition Reviews* **4,** *163 (1946)*) which suggests its application in relief of the diarrhea and leukopenia accompanying radiation sickness.

Inbred Swiss mice were fed on a diet of Purina dog chow and water for two weeks prior to irradiation. In preliminary experiments, conditions were developed which permitted uniform dosage of mice with x-rays. A suitable dose for experimental use was then selected by observing the results of irradiation of two groups of 20 mice with 200 and 500 r, respectively. In mice receiving 200 r, the white blood cell count dropped from an initial level of 17,000 to 8000 on the second day following irradiation, and returned to normal levels on the fifth day. No deaths occurred during this leukopenic phase, the first deaths occurring on the twenty-eighth day after irradiation. Fifty per cent had died on the thirty-ninth day. In the mice receiving 500 r, the white blood cell counts fell to very low levels (1000) and thirty-eight days were required for a return to normal levels. The first deaths in this group occurred within eight days, and 50 per cent had died by the twenty-first day, before the end of the leukopenic phase. For evaluation of the effects of vitamins, an intermediate dosage level, 350 r, was selected, in which the effects of irradiation were of intermediate severity. Control groups and groups receiving 20 micrograms of folic acid (pteroylglutamic acid) orally following irradiation, or 15 micrograms of folic acid intramuscularly for seven days prior to radiation and fifteen days thereafter were compared. No obvious effect of administration of folic acid on the hematologic response was noted, although there was what appeared to be a significant reduction of the number of deaths in the leukopenic phase (from 26 per cent to 2 per cent or less). The average survival time of those animals receiving folic acid intramuscularly was also greatly prolonged.

In the experiments with pyridoxine, three groups of animals were compared. One was the control group, which received no supplementation. A second group received 50 micrograms of pyridoxine hydrochloride by intramuscular injection for seven days prior to irradiation and thirteen days after irradiation. The third group was given the pyridoxine only following irradiation. The results here were marked. The animals which received pyridoxine before irradiation, but not those which received it following irradiation, exhibited greatly prolonged survival times as compared with the control group. In the former group, a 50 per cent mortality rate was obtained only at one hundred days, as compared with sixteen days for the postradiation-treated mice and fourteen days for the irradiated controls. Pyridoxine did not appear to influence the onset or severity of the leukopenic phase; it did reduce the number of deaths in this phase.

Further tests carried out by the same technics confirmed these results; the percentage of animals surviving the radiation treatment after one hundred days was increased from 19 per cent (13 of 68) in the control group to 58 per cent (14 of 24) in the pyridoxine-treated group and 62 per cent (20 of 32) in the folic acid-treated group.

These results suggest the possibilities of further investigations along these lines. Previously published results indicate that desoxycorticosterone acetate (F. Ellinger, *Proc. Soc. Exp. Biol. Med.* **64,** *31 (1947)*) and rutin (P. E. Rekers and J. B. Field, *Science* **107,** *16 (1948)*) also exert a favorable influence on certain aspects of the pathologic

picture which follows radiation injury. It would be of interest to determine the amounts of food and supplementary factors required to show these beneficial effects, and to compare them with customary dietary requirements of the same animals.

## EFFECTS OF FOLIC ACID ANTAGONISTS IN MICE

It is now well established that by suitable structural alterations, synthetic analogues of many vitamins and other growth factors can be prepared which are inhibitory or "toxic" rather than stimulatory to growth of a variety of organisms (*Nutrition Reviews* **4,** *110* (*1946*), **5,** *231* (*1947*)). In most cases such inhibition is alleviated by adequate amounts of the corresponding vitamin or growth factor; i.e., the inhibition of growth is of the competitive variety, and in the presence of a sufficiently large amount of the vitamin, even large amounts of the vitamin antagonist are nontoxic. Although a large number of such vitamin antagonists are now known, the specificity of their action, and the differences in susceptibility of different organisms to their action makes continued investigation of them of great interest. In many instances, their use provides a new means for production of specific vitamin deficiencies, and in some cases the deficiencies thus produced show interesting points of difference from those produced by classic dietary means.

An example of such an inhibitor is the crude reaction mixture containing 7-methylpteroylglutamic acid (7-methylfolic acid), the action of which in mice, rats, and pigs has been previously discussed in this journal (*Nutrition Reviews* **6,** *23* (*1948*)). With each of these animals, the inhibitor produced symptoms reminiscent of folic acid deficiency, all of which were prevented when sufficient folic acid was also present in the ration. The comparative ratios of antagonist to folic acid at which symptoms were produced in various organisms is of interest. For Streptococcus faecalis, 20 to 30 parts of the inhibitor were necessary to suppress growth produced by 1 part of vitamin; for Lactobacillus casei the corresponding ratio was 1000:1. In rats, a somewhat higher ratio, 3000 parts of antagonist to 1 part of vitamin were required to produce signs of acute folic acid deficiency.

Another analogue of this vitamin, 4-aminopteroylglutamic acid (aminopterin) has been synthesized (D. R. Seeger, J. M. Smith, and M. E. Hultquist, *J. Am. Chem. Soc.* **69,** *2567* (*1947*)). This compound, which differs from the parent vitamin in the replacement of the hydroxy group in the 4-position of the pteridine ring by an amino group, is a much more potent antagonist of pteroylglutamic acid than the previously described 7-methylfolic acid. With S. faecalis, for example, a ratio of 8 to 10 parts of 7-methylfolic acid to 1 of folic acid is required to reduce growth of the test organism to one-half maximum (A. L. Franklin, E. L. R. Stokstad, M. Belt, and T. H. Jukes, *J. Biol. Chem.* **169,** *427* (*1947*)); the new antagonist produces the same effect at ratios as low as 0.4 part of antagonist to 1 of folic acid (Seeger *et al.*, *loc. cit.*).

The effect of this inhibitor on the nutrition of the mouse has now been reported by A. L. Franklin, E. L. R. Stokstad, and T. H. Jukes (*Proc. Soc. Exp. Biol. Med.* **67,** *398* (*1948*)). The animals were fed a purified diet which contained 1 per cent of succinylsulfathiazole but no pteroylglutamic acid. In preliminary experiments, it was found that mice died within a few days when fed a diet containing 1 mg. of the inhibitor per kilogram. Consequently, lower levels were fed in the presence and absence of varying quantities of pteroylglutamic acid. At a level of 0.3 mg. per kilogram, aminopterin had a slight tendency to depress values for the hemoglobin and white cell count of blood. This tendency was successfully counteracted by large

amounts of pteroylglutamic acid (1.0 to 10.0 mg. per kilogram). When 1 mg. of aminopterin was present per kilogram of ration, high mortality occurred within one week, despite addition of as much as 100 mg. of pteroylglutamic acid to the ration. At these high levels, the toxicity of the compound does not appear to be alleviated competitively by the corresponding vitamin; it has become instead an irreversible poison. In view of this behavior, it should be noted that although the inhibitory action of the compound for S. faecalis was alleviated by pteroylglutamic acid at all levels tested, the compound was relatively a much more effective inhibitor at high ratios of analogue to vitamin (Seeger *et al., loc. cit.*).

The mechanism by which this compound exerts its irreversible toxic effects is not known. Presumably it combines with enzyme proteins normally combined with pteroylglutamic acid, and does so so tightly that it cannot be displaced by folic acid, even though the latter is added in excess. It is of interest that in this case a structural alteration of a vitamin produced a compound which is highly toxic even when the corresponding vitamin is fed simultaneously and in excess. As such, further investigation of its action should be of great interest.

## METABOLIC EFFECTS IN MAN OF ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPIC HORMONE

Profound metabolic effects involving the metabolism of protein, carbohydrate, cholesterol, sodium, chloride, potassium, water, and uric acid are produced by hormones of the adrenal cortex. In addition, important hematologic effects are produced, as are also effects referable to increased production of sex hormones (androgenic adrenal corticosteroids).

Knowledge concerning the effects in man of injections of purified adrenocorticotropic hormone (ACTH) has had to await the preparation of this material in sufficient quantity to allow performance of such experiments. Larger quantities of such material have become available recently to several investigators. The ACTH used by several groups has been prepared either by the method of C. H. Li, H. M. Evans, and M. E. Simpson (*J. Biol. Chem.* **149,** *413 (1943)*) consisting of salt fractionation, or by the method of G. Sayers, A. White, and C. N. H. Long (*Ibid.* **149,** *425 (1943)*) which employs isoelectric precipitation. Some of the differences in effects observed with the two types of material may be related to their different methods of preparation.

Administration of purified ACTH to man was reported by J. S. L. Browne (*Josiah Macy Jr. Foundation Report, New York, June 11–12, 1943*). In one subject an increased urinary excretion of "cortin-like" substances was observed without any significant change in excretion of 17-ketosteroids. In a second subject the glucose tolerance test shifted toward the diabetic type and was accompanied by glycosuria. Since "cortin-like" substances consist of 11 and 11–17 oxysteroids and since this particular type of adrenal steroid is known to affect the metabolism of carbohydrate ("S" or sugar hormone of Albright) it was assumed that the metabolic effects produced by ACTH would be principally those exerted by increased amounts of 11 and 11–17 oxysteroids.

A large number of steroid compounds have been isolated from the adrenal cortices of animals. The physiologically active ones can be classified according to their major functional effects into one of three main categories:

(1) Adrenal steroids possessing activities of sex hormones, the androgenic ones being indicated by the level of urinary excretion of 17-ketosteroids and being capable of inducing nitrogen retention.

(2) Adrenal steroids which affect the metabolism of protein (negative nitrogen balance) and carbohydrate (decreased utilization), the 11 and 11–17 oxysteroids.

(3) Adrenal steroids which act mainly upon sodium, potassium, and chloride metabolism, the desoxycorticosterone-like steroids.

Could it be that the tropic influence of the pituitary upon the adrenal cortex is limited to stimulating the production of steroids of the second category? If so, what regulates production of the other two types of adrenal corticosteroids?

H. L. Mason *et al.* (*J. Clin. Endocrinology* **8,** *1* (*1948*)) using ACTH prepared by the method of Li *et al.* (*loc. cit.*) gave as much as 100 mg. per day to a normal young woman. A threefold increase in the daily excretion of 17-ketosteroids was observed. Urinary excretion of 11 and 11–17 oxysteroids increased to eight times the base line value. Urinary excretion of pregnandiol and estrogens did not change. Urinary cholesterol fell to below 50 per cent of the base line excretion. Free plasma cholesterol showed a sharp decrease under the influence of ACTH while plasma cholesterol esters did not change significantly.

Electrolyte metabolism appeared to be entirely uninfluenced by ACTH in these studies. Urinary and blood sodium, potassium, chloride, and phosphorus had been done. It is interesting in this connection that these workers noted an increase of 1.5 kg. of body weight on the third day of ACTH injections, that this was associated with pitting edema of the ankles and pretibial regions, and that the extra weight and edema disappeared rapidly upon discontinuation of ACTH.

No significant change in nitrogen equilibrium was noted as the result of administration of ACTH. Creatinine nitrogen remained constant but a definite decrease of urinary creatine nitrogen was observed. No change in carbohydrate metabolism was seen when measured by the glucose tolerance test, but the insulin tolerance test suggested mild resistance to insulin.

No significant hematologic changes were observed in this individual except for the rapid development of an anemia. This is at variance with previous reports in rats (T. F. Dougherty and A. White, *Endocrinology* **35,** *1* (*1944*)) and in man (G. W. Thorn, F. T. G. Prunty, and P. H. Forsham, *Science* **105,** *528* (*1947*)) in which lymphopenia and polymorphonuclear leucocytosis have resulted during administration of ACTH.

In contrast to these results are those reported by Forsham, Thorn, Prunty, and A. G. Hills (*J. Clin. Endocrinology* **8,** *15* (*1948*)) and J. W. Conn, L. H. Louis, M. W. Johnston, and B. Johnson (*J. Clin. Invest.* (*Proc. number, 1948*)). The former group studied one normal man given ACTH for four consecutive days and the latter group observed the metabolic effects produced in 4 normal people (2 men and 2 women) during eight to ten days of ACTH administration. Both groups employed a purified ACTH produced in the Armour Research Laboratories by a modification of the methods of Sayers *et al.* (*loc. cit.*)

Evidence of increased activity of all three types of corticosteroids was obtained by each group:

1. *Adrenal androgenic steroid production.* Forsham's subject showed a mild increase in 17-ketosteroid excretion. Conn's subjects given larger amounts of ACTH showed increases of 300 to 500 per cent above the base line values. One female subject reached a peak excretion of 110 mg. per day of 17-ketosteroids.

2. *Adrenal 11 and 11–17 oxysteroid production.* A 500 per cent increase in urinary excretion of these steroids was observed in Forsham's normal man.

(a) *Uric acid excretion.* Both groups of investigators recorded great increases in the daily urinary excretion of uric acid accompanied by a fall of the level of serum uric acid. Much more uric acid was excreted than could be accounted for on the basis of

increased renal clearance and indicated increased production of this compound.

(b) *Nitrogen balance.* Negative nitrogen balance was not observed in Forsham's normal man. Severe negative nitrogen balance occurred in 2 (one male and one female) of Conn's 4 subjects. A third showed mild loss of body protein while the fourth remained in nitrogen equilibrium. It seems likely that changes in nitrogen balance are determined by the relative amounts of androgenic steroids (protein anabolic) and of 11 and 11–17 oxysteroids (protein catabolic) that are produced in a given individual when his adrenal cortices are stimulated by ACTH.

(c) *Carbohydrate metabolism.* Forsham's normal man showed a mean increase of the fasting blood sugar level during ACTH administration of 26 mg. per cent but no other abnormality of carbohydrate metabolism was observed. No glycosuria occurred.

All of Conn's subjects developed glycosuria during the period of ACTH injections. The amounts of urinary glucose varied between 1.5 g. and 36.0 g. per day. Typically diabetic glucose tolerance curves developed in as short a time as one day and loss of carbohydrate tolerance progressed with the duration of injections. Within a week after cessation of ACTH, carbohydrate tolerance returned to normal (J. W. Conn, L. H. Louis, and C. E. Wheeler, *J. Lab. Clin. Med.* **33,** *651 (1948)*).

(d) *Hematologic changes.* Both groups of workers observed a striking and sustained fall in the circulating eosinophilic leucocytes, a mild lymphopenia, and a marked and sustained increase in the neutrophilic polymorphonuclear cells.

3. *Production of desoxycorticosterone-like steroids.* Forsham's and Conn's groups each observed a prompt and great decrease of urinary excretion of both sodium and chloride upon administration of ACTH. This was accompanied by a mild (Forsham) and marked (Conn) increase of body weight. A transitory increase of urinary potassium was also found by both groups. Serum levels of sodium, chloride, and potassium did not change appreciably, although there was a tendency for serum carbon dioxide combining capacity to be increased at the expense of chloride.

Upon cessation of ACTH administration Forsham *et al.* noted a prompt loss of the sodium and chloride retained during the injection period. Conn *et al.* having administered the material for eight to ten consecutive days observed: (1) marked sodium and chloride retention during the first three to five days with development of edema; (2) a "rebound" to sodium and chloride equilibrium on the fourth to sixth day despite continued ACTH administration, an effect known to occur in normal individuals upon continued administration of desoxycorticosterone; and (3) a great sodium and chloride diuresis upon cessation of injections of ACTH.

In addition, Conn's group demonstrated a great decrease in the concentration of sodium and chloride of the sweat of their normal subjects during the period of ACTH administration. This effect had previously been shown to occur in normals upon administration of desoxycorticosterone (J. W. Conn, M. W. Johnston, and L. H. Louis, *J. Clin. Invest.* **25,** *912 (1946)*).

Thus, virtually every metabolic effect known to be produced by either whole extracts of adrenal cortex or by isolated or synthesized pure adrenal cortical steroids, has been produced in normal individuals by administration of purified pituitary adrenocorticotropic hormone.

## GLUTAMIC ACID AND INTELLIGENCE

Glutamic acid appears to be the only known amino acid to be metabolized by slices of brain tissue (H. Weil-Malherbe, *Biochem. J.* **30,** *665 (1936)*). There is considerable evidence that various enzyme systems are intimately connected with propagation of nerve impulses themselves. Not only is the release of acetylcholine intrinsically connected with the electric changes which take place during the time when the nerves are active but an enzyme called choline acetylase has been isolated which synthesizes acetylcholine (D. Nachmansohn, R. T. Cox, C. W. Coates, and A. L. Machado, *J. Neurophysiol.* **6,** *383 (1943)*; Nachmansohn and Machado, *Ibid.* **6,** *397 (1943)*). Apparently l(+)-glutamic acid acts as a catalyst in the production of acetylcholine and when choline acetylase is inactivated by dialysis, the addition of l(+)-glutamic acid reactivates it (Nachmansohn, H. M. John, and H. Waelsch, *J. Biol. Chem.* **150,** *485 (1943)*). Other compounds, such as cysteine and alanine likewise have some catalyzing effect in the production of acetylcholine but it is not known whether these amino acids are metabolized by the brain.

This background of data led to the investigation of the effects of administration of dl-glutamic acid hydrochloride to patients with petit mal (*Nutrition Reviews* **5,** *174 (1947)*). The drug had no effect on grand mal but did decrease the frequency of slow-wave activity in the electroencephalogram. The changes were striking in the mental and physical alertness of the subjects. Later improvements were found in personality (H. Waelsch and J. C. Price, *Arch. Neurol. Psychiat.* **51,** *393 (1944)*). These suggestive results naturally led to studies with animals. F. T. Zimmerman and S. Ross (*Ibid.* **51,** *446 (1944)*) reported that the maze learning in white rats was significantly enhanced by addition of glutamic acid to their diet and others have confirmed this observation (K. E. Albert and C. J. Warden, *Science* **100,** *476 (1944)*). The results reported by H. C. Hamilton and E. B. Maher (*J. Comp. Psychol.* **40,** *463 (1947)*) on the effect of glutamic acid on rats given the three-table test of "reasoning" were not impressive but warranted further study. In a well controlled study, M. H. Marx (*Ibid.* **41,** *82 (1948)*) was unable to confirm the earlier improvement reported in white rat learning performance after ingestion of glutamic acid. His discussion of potential for performance, learning performance, and learning ability and general activity level gives important points in interpretation of these types of animal data.

These animal studies have in turn led to a clinical trial of glutamic acid in patients (F. T. Zimmerman, B. B. Burgemeister, and T. J. Putnam, *Am. J. Psychiat.* **104,** *593 (1948); Psychosom. Med.* **9,** *175 (1947)*). These investigators present a summary of their research on glutamic acid and its effect on mental function in human subjects. Sixty-nine patients in the original experimental group received treatment with glutamic acid for one year. Included in this group were mentally retarded patients with convulsive disorders and also a group of patients with convulsive disorders and normal intelligence. A report of the effects of treatment with glutamic acid for the first six months appears in an article which is in press and the present report is concerned with the first 30 cases in which a full year of treatment has been completed.

Of these 30 cases, 14 were children or adolescents with convulsive disorders, 7 of whom were mentally retarded. Sixteen had no convulsions but were mentally retarded; this made a total of 23 mentally retarded patients in the experimental group. The control group consisted of 37 mentally retarded patients, both with and without convulsive disorders. These patients had been tested by psychometric tests a number of times over a period of years.

Glutamic acid was administered in gradually increasing doses to the point where increased motor and psychic activity were apparent. It was noted that overdosage produced distractibility, or even insomnia, especially if the last dose was given too close to bedtime. The effective dosage varied from 6 to 24 g. per day in three divided doses. In a few instances up to 48 g. per day were given. On the average, 12 g. per day were sufficient. The material was administered in the form of a tablet, powder, or capsule. No untoward side effects were observed, except occasional gastric distress which could be corrected by discontinuing treatment for a few days.

Before treatment with glutamic acid was started the entire group was given five different psychometric tests. When the effective dosage was established, periodic psychometric examinations were conducted about once a month throughout a period of six months, at the end of which time the same battery of psychometric tests was given to experimental and control groups of patients. The same tests were given again after one year of treatment. The group ranged in age from 5 to 16 years and in intelligence quotient from 38 to 131 at the time of the first test.

In the experimental group the intelligence quotient points gained for the year ranged from 0 to 17. In 10 cases a gain of 12 points or more was shown; in 12 cases a gain of 6 points or more, and the points gained in the remaining 8 cases ranged from 0 to 6. Failure to gain during the experimental period of one year was evident in only one case. On the Stanford-Binet test the group showed an average gain in intelligence quotient of approximately 11 points during one year of treatment with glutamic acid. In terms of mental age this reflected a gain of 24 months, or a rate which is twice as great as that expected in children of average intelligence. In 97 per cent of the cases the second intelligence quotient was higher than the first but in only 53 per cent of the group did the third exceed the second quotient. This observation would suggest that more change took place during the initial period of therapy and that, although the intelligence quotient for the group was still rising at the end of one year, the intelligence quotient appeared to be rapidly approaching an upper limit. The same trend was apparent in the results of performance tests.

The underlying physiologic mechanisms which are responsible for the reported effects of glutamic acid on intelligence are not entirely clear. It is admitted that there is some physiologic basis which would cause one to suspect that glutamic acid in some way is perhaps related to the formation of acetylcholine and that this, in turn, has something to do with the biochemical level of intelligence. The authors point out that since administration of glutamic acid can enhance the capacity of individuals to act intelligently and can increase maze learning in the white rat, one must be careful in defining intelligence in an individual as inherent and unchangeable. Perhaps at this time the greatest value of this type of study is that it stimulates further investigations in the field of nerve and brain metabolism. Certainly, when the final answers are known, changes in mental function will probably resolve to alterations in biochemical equations and advances in this direction should be greatly encouraged.

## PLASMA AND LIVER FAT CONTENT IN CIRRHOSIS

Among the many nutritional and metabolic abnormalities exhibited by patients with liver disease are those concerned with lipids. In this regard G. H. Stueck *et al.* (*Am. J. Med.* **5**, *188* (*1948*)) have reported the plasma and liver lipid concentration in 21

patients with liver disease of whom 19 had cirrhosis.

The plasma lipids were determined, sometimes several times, at intervals varying from two hundred and fifty-four days before death to the day of death. Liver lipids were determined on specimens obtained at the autopsies which were performed on all cases. The analyses on both plasma and liver included total lipids, total fatty acids, neutral fat, phospholipids, free cholesterol, and cholesterol esters.

The nutritional status of the patients varied greatly: it was considered to be good in 3, fair in 2, poor in 6; 6 patients were emaciated while 3 were obese. Most of the patients were jaundiced; 16 had ascites; and liver function tests, performed on most, showed evidence of severe functional hepatic impairment.

The patients were divided into three groups: 7 with an increase in plasma lipids, 5 with an increase in liver lipids, and 12 in whom the only change was in the relation of free total cholesterol in the plasma. There was no consistent relation between the plasma and liver lipids. Thus of the patients in group I, only 3 had an increase in liver lipids. In group II, the increases in liver lipids were very large, and pathologically all but one showed extensive fatty changes. In no instance did the phospholipids increase in the livers of these patients, but fatty acids and neutral fat were far above normal.

The 12 patients in group III had as their only lipid abnormality an increase in the ratio of free to total plasma cholesterol above the authors' normal of 29 per cent, in fact, all but one of the patients in the entire series showed this abnormality. The latter patient had an infiltrating lymphosarcoma as well as underlying cirrhosis. The authors believe that the diagnosis of cirrhosis of the liver should not be made if the ratio of free to total cholesterol is within normal limits.

Vitamin A and carotene were determined in the plasma and livers of 11 cases. The plasma vitamin A values were all below normal, most far below. The plasma carotene levels were likewise reduced but less than in the case of vitamin A. There was great variation in the vitamin A content of the livers, to a less extent for carotene. Normal liver contents of these substances are not given. There appeared to be little if any correlation between the blood and liver contents, although the patient with the highest plasma vitamin A and carotene concentration also had one of the highest liver contents of both substances. Pathologically this patient had a liver cell carcinoma superimposed upon a multilobular cirrhosis.

The lack of correlation of plasma and liver lipids is interpreted by the authors as evidence that several factors are at work. Thus the nutritional status of the patient may play a role, as well as the functional impairment of liver phosphorylation and, at least in the early stages, the pathologic accumulation of fat in the liver.

The most consistent finding in the study was the invariable inversion of the ratio of free to total cholesterol in every case of cirrhosis of the liver.

## NUTRITIONAL REQUIREMENTS OF THE FOX AND MINK

The development of commercial fox and mink farms has been the stimulus for the investigation of the nutritional requirements of these species. It is impractical to supply the types of food selected by the animals in their natural wild state and, as a result, it has been the usual custom to substitute a dietary composed of relatively inexpensive materials such as horse meat, fish, cereals, and vegetables. The composition of the rations has been determined largely by experience gained by trial and error. In at

least one instance the procedure has added to biochemical knowledge. So-called Chastek paralysis, which has been observed in foxes fed a food mixture containing raw fish, has been demonstrated to be due to a deficiency of thiamine resulting from the inactivation of dietary thiamine by a substance present in certain types of uncooked fish (*Nutrition Reviews* **1,** *61, 403 (1942–43)*).

The use of a dietary composed of known purified nutrients has not been successful on fox and mink farms or in the laboratory because both species require one or more unidentified food factors in addition to the recognized vitamins (A. E. Schaefer, C. K. Whitehair, and C. A. Elvehjem, *Arch. Biochem.* **12,** *349 (1947)*). When fox pups and adults were fed a purified ration containing all of the crystalline vitamins, the young gained weight for fifteen to twenty weeks and the older animals maintained body weight for thirty-two to forty weeks before general failure occurred. The deficiency syndrome which was prevented by supplemental whole liver was characterized by anorexia, anemia, loss of weight, and death. In the absence of folic acid the symptoms appeared much earlier and prompt restoration followed its administration. However, the inclusion of folic acid in the diet failed to prevent the later appearance of the severe deficiency state.

These results have been extended in a subsequent report by the same authors (*J. Nutrition* **35,** *147 (1948)*). Weight loss, anemia, depigmentation and matting of fur, anorexia and, in some cases, paralysis occurred in both fox pups and in adults on a purified diet containing folic acid. In fatal cases the liver was yellow, enlarged, and fatty and the kidneys appeared pale-colored and degenerate. These evidences of a dietary deficiency were not prevented by a doubling of the vitamin supplement, by a refined preparation of the antipernicious anemia factor, by increased protein (casein), or by the methanol-insoluble residue of whole liver. Restoration of weight and of hemoglobin occurred after supplementation of the diet with raw whole milk (50 ml. daily), with fresh liver (10 per cent of diet), or with a 60 per cent methanol extract of liver. The results indicated that at least two factors were present in liver and in milk, one concerned with growth and maintenance of weight and one concerned with hemoglobin production. Dried brewers' yeast supplied the growth and weight factor but was without effect on the anemia. Whether or not the unidentified factors are related to folic acid, which delays the onset of anorexia and anemia, or to the new substance, vitamin $B_{12}$ (*Nutrition Reviews* **6,** *245, 291 (1948)*), remains to be determined.

Results similar to these have been obtained in mink kits and adults which also show anorexia, growth failure, anemia, and paralysis of the hindquarters on purified diets containing the known vitamins (A. E. Schaefer, S. B. Tove, C. K. Whitehair, and C. A. Elvehjem, *J. Nutrition* **35,** *157 (1948)*). Whole liver was completely effective in alleviating these symptoms.

The observation that the fox and the mink do not require a dietary source of ascorbic acid has been confirmed by C. F. Bassett, J. K. Loosli, and F. Wilke (*J. Nutrition* **35,** *629 (1948)*). These authors found, however, that supplements of ascorbic acid appeared to decrease the vitamin A requirement and that a similar effect resulted when the diet contained 50 per cent of cooked potatoes. In these experiments fox pups and mink kits were fed a basal mixed diet which contained only traces of vitamin A and carotene and which was low in ascorbic acid. Groups were given supplements of vitamin A, with and without added ascorbic acid (20 mg. per pound of feed). Signs of vitamin A deficiency, such as head cocking, whirling and weaving, or staggering gait, appeared in 38 per cent of the foxes on the unsupplemented basal ration. None of these symptoms was evident in the pups receiving the ascorbic acid supplement. Furthermore, the level of

vitamin A in the serum and in the liver was higher in the animals on the vitamin A-free diet when the diet contained ascorbic acid. In both series there was a consistent increase in the vitamin A content of the blood and liver as the dietary supply of vitamin A was increased. Diets containing 50 I.U., or more, of vitamin A per pound were satisfactory for growth and for prevention of deficiency symptoms.

In the case of the mink kits, specific signs of vitamin A deficiency did not appear, indicating either that the requirement was less than that of young foxes or that the original liver storage was greater. Significantly more vitamin A was found in the serum and liver of animals on the vitamin A deficient ration when ascorbic acid was fed. Mortality was high on all rations, suggesting that the basal diet might have been less adequate for the mink than for the fox. The inclusion of 50 per cent of cooked potatoes in the basal diet yielded results similar to those resulting from supplementation with ascorbic acid. In general, the sparing action on vitamin A was greater and the mortality was lower on the potato diets.

The experiments of Bassett *et al.*, add to the evidence of an interrelation between vitamin A and ascorbic acid. The level of serum ascorbic acid and the rate of excretion in the urine of rats vary with the dietary intake of vitamin A (*Nutrition Reviews* **1,** *48 (1942)*). Similar effects have been demonstrated in calves (P. D. Boyer *et al.*, *J. Nutrition* **23,** *525 (1942)*). The explanation of a vitamin A to ascorbic acid relation is unknown unless it is the indirect effect of a poor nutritional state on the synthesis of ascorbic acid in vitamin A deficient animals. It is possible that the vitamin A to ascorbic acid relation may have the same basis as that suggested for the sparing action of tocopherols on vitamin A, viz., an antioxidant effect (*Nutrition Reviews* **3,** *17 (1945);* **4,** *324 (1946);* **5,** *265 (1947)*).

## BIOCHEMICAL FUNCTION OF VITAMIN E

The most prominent results of experimental vitamin E deficiency in animals have centered around resorption sterility in the female rat and muscular dystrophy in various species. From the biochemical standpoint the antioxidant properties of the tocopherols seem particularly pertinent to a possible physiologic function, and much evidence has been gathered to support this contention. Parallel with such studies have been the observations that dystrophic muscles from vitamin E deficient animals show an increased oxygen consumption which could be decreased by the addition of vitamin E either *in vivo* or *in vitro* (O. B. Houchin and H. A. Mattill, *J. Biol. Chem.* **146,** *309 (1942)*). Although the latter observations might be interpreted in the broadest sense as another "antioxidant" function of vitamin E, they seem more logically (from the biochemical standpoint) to constitute an alternative function of vitamin E. It has not been necessary thus far however, to consider more than a single biochemical function of a vitamin to explain the pathology of its deficiency, as varied as the pathology may at first seem to appear. On the other hand, it is too often forgotten that vitamins might display certain characteristics as chemical substances which are not necessarily connected with their physiologic function. Several recent studies on the action of vitamin E on muscle enzyme systems may be pertinent to the understanding of the biochemical function of this vitamin.

The antioxidant properties of vitamin E can explain many of its physiologic effects. Thus the sparing action of tocopherols on carotene and vitamin A (*Nutrition Reviews* **3,** *17 (1945)*) and the decreased stability of

body fat in vitamin E deficiency (*Ibid.* **5**,*243* (*1947*)) seem definitely related to this property. There is much scattered evidence that the quantity and type of fat in the diet affects the vitamin E requirement of the animal. Resorption sterility in vitamin E deficiency has been suggested as due to the toxic effect of peroxides formed because of the lack of the physiologic antioxidant action of the vitamin (*Ibid.* **3**, *17* (*1945*)). The acid-fast pigment noted in the musculature of vitamin E deficient rats may be in the nature of peroxidized and polymerized unsaturated fatty acids, hence this pathologic finding also appears related to the antioxidant function of the vitamin (L. J. Filer, R. E. Rumery, and K. E. Mason, "Biological Antioxidants," *p. 67, Transactions of the First Conference, Josiah Macy Jr. Foundation, New York (Oct. 10–11, 1946)*). The antioxidant functions of vitamin E have been extensively reviewed and discussed in the reference just cited. Can the muscular dystrophy of vitamin E deficiency also be related to the antioxidant property of this vitamin?

Several investigators have noted an exceptionally high oxygen consumption in the dystrophic muscles of vitamin E deficient animals, and Houchin and Mattill (*Nutrition Reviews* **1**, *308* (*1943*)) showed that this could be promptly reduced either by injection of alpha-tocopherol phosphate into the animal or by addition to the medium in which the muscle preparation was respiring. The effect was believed to be traced to the succinic oxidase system. Since then several workers have tried to localize this biochemical effect, and explanations, although not simple, have been offered. In a series of papers, W. M. Govier and co-workers have shown that alpha-tocopherol phosphate inhibits the succinic oxidase system of normal muscle (Govier, V. Bergmann, and K. H. Beyer, *J. Pharmacol. Exp. Therap.* **85**, *143* (*1945*)), then postulated (Govier, N. Yanz, and M. E. Grelis, *Ibid.* **88**, *373* (*1946*)) and provided proof that the mechanism involved is directly that of inhibiting DPN-ase, the enzyme which hydrolyzes the coenzyme DPN (diphosphopyridine-nucleotide) (Govier and N. S. Jetter, *Science* **107**, *146* (*1948*)). The effect on this enzyme was determined by measuring directly the change in DPN concentration before and after incubation. M. E. Spaulding and W. D. Graham (*J. Biol. Chem.* **170**, *711* (*1947*)) also found that alpha-tocopherol inhibits the action of DPN-ase.

The coenzyme DPN is normally required for the conversion of malate to oxalacetate, and the latter metabolite is a strong inhibitor of succinic dehydrogenase. It may thus be pictured that the added tocopherol, by inhibiting the DPN-destroying enzyme, preserves DPN and thus increases the oxalacetate concentration in the muscle preparation. Oxalacetate then inhibits succinic dehydrogenase. In the absence or deficiency of tocopherol it may therefore be presumed that DPN is more readily destroyed, there is a lack of oxalacetate formation, and succinic dehydrogenase activity is not inhibited and is thus excessively high.

S. R. Ames (*J. Biol. Chem.* **169**, *503* (*1947*)) made a fairly extensive study of the tocopherol-succinic oxidase relations in homogenized rat muscle preparations. He also noted the inhibiting effect of alpha-tocopherol phosphate on succinic acid oxidation, but attributes the primary effect of tocopherol to formation of an insoluble salt with calcium. Since calcium is normally an activator for the above mentioned DPN-ase, calcium removal effectively causes an inhibition of DPN-ase. The consequences of DPN-ase inhibition have already been outlined. The possible involvement of calcium further complicates an already difficult picture, but may have some physiologic interest. Thus S. Morgulis and W. Osheroff (*Ibid.* **124**, *767* (*1938*)) noted some time ago that dystrophic muscle in vitamin E defi-

ciency showed an average increase of calcium of 500 per cent over normal muscle. In view of this excessive amount of calcium, its relation to tocopherol, and the direct effect of calcium on enzymatic activities, Ames suggests that the abnormally high oxygen intakes observed in dystrophic muscle may be due to this excessive deposition of calcium. Ames has also called upon this high level of calcium in dystrophic muscle to explain the results of D. H. Basinski and J. P. Hummel (*Ibid.* **167,** *339 (1947)*) who were unable to find an abnormally high succinic dehydrogenase activity in dystrophic muscle. In the latter experiment sufficient calcium was added to the assay system to produce maximal activation, while in earlier experiments the different levels of calcium in the normal versus dystrophic muscles themselves greatly affected the relative succinic dehydrogenase activities.

It cannot be inferred from the studies reviewed that the action of tocopherol with calcium or its inhibiting action on DPN-ase bears any relation to the development of muscular dystrophy in vitamin E deficiency. These actions may be related only to the incidental chemical properties of tocopherol, causing these effects on the enzyme systems in a tissue homogenate. With the antioxidant properties so well substantiated as the probable basis for the action of this substance as a vitamin, it is difficult to attribute these very indirect actions of tocopherol to its vitamin function. More studies should be made however on the assay of oxidative enzyme systems in vitamin E deficient muscle as contrasted to normal.

In regard to the latter point, O. B. Houchin ("Biological Antioxidants," *p. 60, Transactions of the First Conference, Josiah Macy Jr. Foundation, New York (Oct. 10–11, 1946)*) has continued studies of the effect of alpha-tocopherol phosphate on enzyme systems. Using brain tissue, he reports that tocopherol inhibits the reduction of cytochrome-c by ascorbic acid. It is implied that this action occurs in all biological oxidations involving cytochrome-c and that "alpha-tocopherol (vitamin E) in this regard must act as a 'biological antioxidant' through an antireductant action." From what is known about the reduction of cytochrome-c and the action of a typical antioxidant in fat autoxidation, it seems difficult to classify such an action of alpha-tocopherol as "antioxidant." Nevertheless, this is a step in the right direction toward unifying the apparently different pathologic changes of vitamin E deficiency. It seems possible that muscular dystrophy in this deficiency is due to a toxic action of fat peroxides; on the other hand, few have considered that the lack of vitamin E might markedly affect fat oxidation in the tissues. This is of course due largely to the fact that little of the mechanism of fatty acid oxidation in muscle is known except that it does occur. Studies in this basic phase of biochemistry may provide a new understanding of vitamin E function.

## RELATION BETWEEN ASCORBIC ACID AND PHAGOCYTIC ACTIVITY OF LEUCOCYTES

That the concentration of blood ascorbic acid is higher in the white cells than in the red cells or plasma is well known, and it has been shown that the white cell-platelet layer is the last fraction of the blood to be depleted of its vitamin content.

The significance of this high concentration of ascorbic acid in leucocytes is not clear. Possibilities come to mind however, for example, the fact that the cellular response to intraperitoneal irritation is poor in scorbutic guinea pigs (D. Perla and J. Marmorston, *Arch. Path.* **23,** *543 (1937)*). Again, various workers as, for example,

E. Cottingham and C. A. Mills (*J. Immunology* **47**, *493* (*1943*)) have found an impairment of the phagocytic activity of leucocytes from donors low in ascorbic acid. W. J. Nungester and A. M. Ames (*J. Infect. Dis.* **83**, *50* (*1948*)), in studying the fundamental mechanisms governing phagocytosis, observed that in guinea pigs deficient in ascorbic acid, the peritoneal exudate was blood tinged and contained few white cells which, in addition, were unusually fragile. This observation, in addition to the data in the literature already cited, led these workers to make a special study of the relation of ascorbic acid and phagocytic activity.

Guinea pigs were maintained on a standard complete diet furnishing all essentials except ascorbic acid. When manifestations of scurvy developed, a control group was given ascorbic acid. White cells and serum were obtained from these two groups of animals. The white cells were obtained by inducing exudation into the peritoneal cavities of the donor animals by injecting Locke's solution containing 0.1 per cent of glucose intraperitoneally. Appropriate measures were taken to collect leucocytes for study of phagocytosis and for measurement of ascorbic acid content. As antigen in the phagocytic tests, a bacterial suspension was employed containing a beta hemolytic streptococcus killed by heat.

Exudates from deficient animals were strikingly lacking in white cells and frequently the material was made even less satisfactory for phagocytosis studies by the presence of large numbers of red blood cells. Such hemorrhage was never found accompanying exudates of which the ascorbic acid content was above 0.45 mg. per 100 ml. The cells from ascorbic acid-low donors, moreover, proved to be singularly difficult to prepare for use. When serum was not used in the phagocytic systems, the ease of rupture of the cells during incubation was found to bear an inverse ratio to the ascorbic acid level of the exudate. The rupturing was especially marked when the ascorbic acid level was between 0.15 and 0.60 mg. of the vitamin per 100 ml. of exudate. Serum in the phagocytic systems not only promoted activity but was also found to have a protecting action for the cells. The quantity of serum necessary to prevent rupture of the cells varied inversely with the ascorbic acid content of the cells.

Thus, from these carefully carried out studies, it would seem that there is a relation between the ascorbic acid content of exudative polymorphonuclear leucocytes from guinea pigs and the fragility and the phagocytic activity of these cells. The level of ascorbic acid metabolism of the donor animals and the quantity and quality of the exudate obtained from injection of an irritating substance into the peritoneal cavity, also seemed to be related. Clearly, these interesting observations deserve extension and amplification.

## THE HYPERGLYCEMIC EFFECT OF THIOUREA AND RELATED COMPOUNDS

K. P. DuBois, L. W. Holm, and W. L. Doyle (*Proc. Soc. Exp. Biol. Med.* **61**, *102* (*1946*)) and DuBois, R. G. Herrmann, and W. F. Erway (*J. Pharmacol. Exp. Therap.* **89**, *186* (*1947*)) studied the effects on carbohydrate metabolism of alpha-naphthylthiourea (ANTU), allylthiourea, phenylthiourea, and thiourea. ANTU and phenylthiourea exerted a marked hyperglycemic effect in rats when administered in amounts as small as 10 mg. per kilogram of body weight. Thiourea and allylthiourea were found capable of a similar activity but much larger doses were required. Although an increased amount of epinephrine could not be detected in the blood, it was found

that adrenal demedullation completely prevented the hyperglycemic effect of these compounds. The hyperglycemia was associated with depletion of liver glycogen. There was no evidence of decreased carbohydrate combustion to account for the elevated blood sugar levels. Either insulin or ergotamine proved to be antagonistic to the hyperglycemia.

A similar investigation of dithiobiuret (DBT), a compound related chemically ($NH_2 \cdot C(=S) \cdot NH_2C(=S) \cdot NH_2$) to the compounds mentioned above has been reported by S. Seifter, D. M. Harkness, E. Muntwyler, and J. Seifter (*J. Pharmacol. Exp. Therap.* **93,** *93 (1948)*). This compound, too, produced hyperglycemia in rabbits. The material was given subcutaneously at three levels of dosage: 7, 40, and 100 mg. per kilogram of body weight. Single injections at the lower dosage levels produced no hyperglycemia, but a single injection of 100 mg. per kilogram produced intense and prolonged hyperglycemia (260 mg. per cent five hours after injection). Prolonged administration at the lower dosages sometimes produced a moderate elevation of the blood sugar (220 mg. per cent after twelve days of injection at the 7 mg. level).

Rabbits receiving the 100 mg. dose of DTB exhibit a flaccid paralysis within several hours after administration of the drug. This phenomenon was reported by E. B. Astwood (*J. Pharmacol. Exp. Med.* **78,** *79 (1943)*) and Astwood *et al.* (*Science* **102,** *106 (1945)*). Seifter *et al.* (*loc. cit.*) found that prolonged administration at the 7 mg. level invariably produced paralysis but only occasionally hyperglycemia. Thus, they concluded that the paralysis and the hyperglycemia produced by DTB are not mutually dependent. Analyses of skeletal muscles for water, chloride, sodium, potassium, phosphorus, and creatine showed no abnormality.

Intravenous glucose tolerance done upon rabbits paralyzed by administration of 7 mg. per day of DTB showed a somewhat higher curve, but the rate of disappearance of glucose from the blood was thought not to be significantly different from the controls. Tolerance tests were apparently not performed upon animals given sufficient DTB to produce hyperglycemia.

Examination of thyroid, thymus, and adrenal glands of animals dying from DTB was believed to show the changes characteristic of the alarm reaction (H. Selye, *J. Clin. Endocrinology* **6,** *117 (1946)*). The authors suggest that the hyperglycemia is due to epinephrine since it is antagonized by insulin and ergotamine and since DTB does not produce it in adrenal-demedullated animals.

In view of the facts (1) that epinephrine stimulates adrenal cortical function via increased production of pituitary adrenocorticotropic hormone (C. N. H. Long, "Recent Progress in Hormone Research," *Vol. I, Academic Press, Inc., New York (1947)*); (2) that administration of pituitary adrenocorticotropic hormone produces hyperglycemia (J. W. Conn, L. H. Louis, and C. E. Wheeler, *J. Lab. Clin. Med.* **33,** *651 (1948)*); and (3) that the alarm reaction (evidence of which was said to be found in the DTB treated animals) is the result of increased activity of pituitary adrenocorticotropic hormone (Selye, *loc. cit.*) it seems likely that the change in carbohydrate metabolism produced by thiourea and its derivatives is of adrenal cortical origin. Epinephrine appears to be the trigger, in this instance, which fires the pituitary-adrenal axis. It is probable that this mechanism can be set in motion by a great variety of noxious stimuli although all are not mediated via the adrenal medulla. The phenomenon warrants increased attention by all who attempt to interpret metabolic data.

# NOTES

## Letter to the Editor

Dear Sir:

In a "Letter to the Editor" (*Nutrition Reviews* **6,** *191 (1948)*) concerning our observations on the impaired antibody response of pantothenic acid and riboflavin deficient rats (A. E. Axelrod, B. B. Carter, R. H. McCoy, and R. Geisinger, *Proc. Soc. Exp. Biol. Med.* **66,** *137 (1947)*), H. C. Stoerk states . . . "the effect of starvation to the extent present in their riboflavin and pantothenic acid deficient animals was not controlled by paired feeding."

Obviously, Dr. Stoerk overlooked the paired fed controls for a group of riboflavin deficient animals presented in our publication. The antibody response of these controls (final weight = 88 g.) was greater than that of either the riboflavin deficient (final weight = 68 g.) or pantothenic deficient rats (final weight = 97 g.). Since both the food intakes and the final weights of the pantothenic acid deficient rats were greater than those of these control animals, the impaired antibody response of the pantothenic acid deficient rats cannot be attributed to inanition *per se*. The lack of correlation between the degree of inanition and antibody response is further emphasized by the fact that riboflavin deficient rats show a greater antibody response than either pantothenic acid or pyridoxine deficient rats despite the fact that they are in a poorer nutritional state as judged by weight and symptomatology.

These observations justify the conclusion that pantothenic acid and, probably, riboflavin were directly concerned with the establishment of the high antibody titers observed in our experiments.

A. E. Axelrod
B. B. Carter
R. H. McCoy
Institute of Pathology, The Western Pennsylvania Hospital; and Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania

## Survey of Nutrition Research

A Survey of Food and Nutrition Research in the United States has been published by the Food and Nutrition Board of the National Research Council. The data were collected during 1947 and provide a comprehensive listing of projects, laboratories, and personnel working or qualified to work on food and nutrition problems. The report is well organized and should prove to be extremely useful. Copies may be obtained by writing to the Food and Nutrition Board, National Research Council, 2101 Constitution Ave., Washington, D. C. Price: $1.00.

## Nutrition Report

The report of Dr. C. G. King, Scientific Director of the Nutrition Foundation, Inc., for the year ending June 30, 1948 is available for distribution. Copies may be obtained by sending a request to the Foundation's office in the Chrysler Bldg., New York City. In addition to Dr. King's report a complete summary of the appropriations for grants-in-aid and a list of published papers arising from grants from the Foundation are given.

## Protein Hydrolysate for Peptic Ulcer

Therapeutic measures for peptic ulcer are numerous and of great variety, probably indicating that none is perfect. Each new therapy is met with a wave of enthusiasm when introduced, which usually abates as more experience and mature judgment are focused upon its effectiveness. One of the most recent regimens for the management of patients with peptic ulcer makes use of frequent feeding of a protein hydrolysate. The amphoteric qualities of the hydrolysate were thought to be effective in neutralizing gastric acidity, and, in addition, the material presumably acted as a good source of protein for the body. Reports of this therapy, largely enthusiastic, were reviewed in this journal (*Nutrition Reviews* **5,** *282 (1947)*), but caution in interpreting these preliminary results was suggested.

E. E. Woldman *et al.* (*J. Am. Med. Assn.* **137,** *1289* (*1948*)) have contributed studies on the action of protein hydrolysates on gastric physiology which lead to further caution in accepting the enthusiastic preliminary trials. The subjects were studied while fasting. The state of health of these subjects is not made clear, although it is stated that the study was not limited to patients with gastrointestinal disease.

In the first part of the study the effect was observed of drinking 25 g. of protein hydrolysate dissolved in 250 ml. of water on the fasting and subsequent gastric acidity in 10 subjects. In the gastric juice specimen obtained fifteen minutes after the hydrolysate the free acidity had fallen from the fasting level (in all but one instance free acid was present) to zero. The period of neutralization lasted from thirty to sixty minutes. There followed a rise in free acid concentration so that in 7 of the 10 subjects its concentration was above fasting in from ninety to one hundred and five minutes after hydrolysate administration. In many instances it was considerably higher.

From these studies it might be thought that if the hydrolysate were administered at frequent intervals a better result might be achieved. Such was not the case. Hourly administration of 25 g. of hydrolysate for three hours to one patient resulted in progressively higher free acid concentration after the neutralizing effect was gone. A final concentration almost twice the fasting value resulted. In addition to failing to maintain neutralization of the free acid, hydrolysate appeared to reduce significantly gastric emptying time.

Twenty-two patients with peptic ulcer were given a continuous intragastric drip of the hydrolysate. Despite this, random gastric specimens always contained free acid. Furthermore, it was the impression of the authors that clinical improvement was slower than with standard forms of therapy.

The nutritional value of protein hydrolysates has been considered by many as an added benefit to the management of patients with peptic ulcer. However, Woldman *et al.* were unable to demonstrate protein deficiency in most of their patients and concluded that this additional source of protein was unnecessary. It is notoriously difficult to determine an individual's state of nutriture with regard to protein. The authors do not state their criteria, except to say that plasma proteins were not reduced. They also observe that with normal serum proteins and a positive nitrogen balance more protein would not aid in healing the ulcer. Unfortunately, neither the plasma protein concentration nor the finding of a positive nitrogen balance gives more than a hint as to the state of the body with respect to protein. Nevertheless, even if the hydrolysate were to assist in healing by furnishing additional protein to the body, the failure to maintain low free gastric acid, and particularly the rebound phenomenon observed, put hydrolysate therapy on much less firm ground than the previous clinical reports would indicate.

## *BOARD OF TRUSTEES*

*Karl T. Compton, *Chairman*
*President, Massachusetts Institute of Technology*

*Cason J. Callaway, *Vice Chairman*
*Hamilton, Georgia*

Joseph F. Abbott, *President,*
*American Sugar Refining Co.*
Paul Armstrong,
*General Manager,*
*California Fruit Growers Exchange*
A. C. Benjamin, *Manager,*
*"Junket" Brand Foods,*
*Chr. Hansen's Laboratories*
Carlyle H. Black,
*Executive Vice President,*
*American Can Co.*
*F. G. Boudreau, *Chairman,*
*Food and Nutrition Board,*
*National Research Council*
R. K. Brodie, *Vice President,*
*The Procter & Gamble Company*
Frank E. Buck, *President,*
*Golden State Co., Ltd.*
Ellsworth Bunker, *President,*
*National Sugar Refining Co.*
*O. C. Carmichael, *President,*
*The Carnegie Foundation for the Advancement of Teaching*
*Rev. John J. Cavanaugh,
*President, Notre Dame University*
*W. C. Coffey,
*President Emeritus,*
*University of Minnesota*
Carle C. Conway, *Chairman,*
*Continental Can Co., Inc.*
George H. Coppers, *President,*
*National Biscuit Company*
E. B. Cosgrove, *President,*
*Minnesota Valley Canning Co.*
Daniel W. Creeden,
*President,*
*Libby, McNeill & Libby*
*Charles Wesley Dunn,
*Member of New York Bar*
A. W. Eames, *President,*
*California Packing Corp.*
George A. Eastwood,
*Chairman,*
*Armour and Company*
Edgar J. Forio,
*Vice President,*
*The Coca-Cola Company*
Clarence Francis, *Chairman,*
*General Foods Corp.*
*E. B. Fred, *President,*
*University of Wisconsin*
Talbot O. Freeman,
*Vice President,*
*Pepsi-Cola Company*
Frank Gerber, *Chairman,*
*Gerber Products Co.*
*Rufus C. Harris, *President,*
*Tulane University*
Henry J. Heinz II, *President,*
*H. J. Heinz Co.*
John Holmes, *President,*
*Swift & Company*
*Charles Glen King, *Scientific Director of the Nutrition Foundation, Inc.*
James E. Knox,
*General Manager,*
*Chas. B. Knox Gelatin Co., Inc.*
J. Preston Levis, *President,*
*Owens-Illinois Glass Co.*
C. R. Lindback, *President,*
*Abbott's Dairies, Inc.*
K. D. Loose, *Vice President,*
*Sunshine Biscuits Inc.*
Arthur McCallum, *President,*
*Flako Products Corp.*
Charles P. McCormick,
*President,*
*McCormick & Company*
James McGowan, Jr.,
*President,*
*Campbell Soup Co.*
J. S. McLean, *President,*
*Canada Packers, Limited*
Armand May, *Chairman,*
*American Lecithin Co., Inc.*
John T. Menzies, *President,*
*The Crosse & Blackwell Co.*
Joel Mitchell, *President,*
*Standard Brands, Inc.*
William T. Nardin, *Vice President and General Manager,*
*Pet Milk Company*
Victor T. Norton, *Vice President and General Manager,*
*American Home Foods, Inc.*
Walter P. Paepcke, *Chairman,*
*Container Corp. of America*
*Thomas Parran,
*Surgeon General,*
*U. S. Public Health Service*
Russell G. Partridge, *Representing United Fruit Company*
D. B. Peck, *President,*
*Bowman Dairy Company*
Mario Peruzzi, *President,*
*Planters Nut & Chocolate Co.*
Philip W. Pillsbury,
*President,*
*Pillsbury Mills, Inc.*
R. A. Robertson,
*Vice President,*
*George Weston, Limited*
Morris Sayre, *President,*
*Corn Products Refining Co.*
Otto Schnering, *President,*
*Curtiss Candy Company*
*George A. Sloan, *President of the Nutrition Foundation, Inc.*
B. E. Snyder,
*President and Treasurer,*
*R. B. Davis Company*
Norman W. Stewart,
*President,*
*E. Pritchard, Inc.*
Vernon Stouffer, *President,*
*The Stouffer Corporation*
R. Douglas Stuart,
*Vice-Chairman,*
*The Quaker Oats Co.*
G. Cullen Thomas,
*Vice President,*
*General Mills, Inc.*
L. A. Van Bomel, *President,*
*National Dairy Products Corp.*
W. H. Vanderploeg, *President,*
*The Kellogg Company*
J. V. Vosburgh, *Vice President,*
*Beech-Nut Packing Company*
L. A. Warren, *President,*
*Safeway Stores, Inc.*
*Ray Lyman Wilbur,
*Chancellor,*
*Stanford University*
*M. L. Wilson, *Chief,*
*Nutrition Programs,*
*U. S. Department of Agriculture*
Charles L. Wirth, *President,*
*Red Star Yeast and Products Co.*
*Stephen S. Wise, *Rabbi,*
*Free Synagogue*
C. I. Wood, *Vice President,*
*Thomas J. Lipton, Inc.*
Harry W. Zinsmaster,
*President,*
*Zinsmaster Baking Co.*

Ole Salthe, *Secretary*

* Asterisk (*) indicates Trustee as representative of the public.

# THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.

A leaflet based on articles appearing currently in NUTRITION REVIEWS and entitled "Current Research in the Science of Nutrition" is distributed free of charge by the Nutrition Foundation each month. The vocabulary of the leaflet is adapted for lay reading and the subject matter is restricted to clearly established findings of practical interest. Those who wish to have their names placed on the mailing list should write directly to the Nutrition Foundation, Inc., Chrysler Building, New York City.